



## Environmental Science and Technology Department annual report 1990

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**Risø-R-588**

# **Environmental Science and Technology Department Annual Report 1990**

**A. Jensen, J. Helms Jørgensen, O.J. Nielsen, K. Nilsson  
and A. Aarkrog**

**Risø National Laboratory, Roskilde, Denmark  
March 1991**

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**Abstract.** Selected activities of the Environmental Science and Technology Department during 1990 are presented. The research in the department is predominantly experimental, and the research topics emphasized are introduced and reviewed in eight chapters: 1. Introduction, 2. The Atmospheric Environment, 3. Plant Genetics and Biology, 4. Nutrient Efficiency in Plant Production, 5. Chemistry of the Geosphere, 6. Ecology and Mineral Cycling, 7. Other Activities, 8. Large Facilities.

The department's contribution to national and international collaborative research programmes is presented together with information about large facilities managed and used by the department as well as activities within education and training.

Lists of scientific and technical staff members, visiting scientists, Ph.D. students, publications, lectures and poster presentations are included in the report.

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# Contents

<b>1 Introduction</b>	<b>5</b>	Denitrification	23
<b>2 The Atmospheric Environment</b>	<b>7</b>	4.2 Root-Microbe Symbioses	24
2.1 Introduction	7	Symbiotic N Fixation	24
2.2 The Gas Phase	7	Mycorrhizas and Plant Nutrition	25
A New Group of Chemicals	7	4.3 Influence of Plant Variety	
Halogenated Organic Compounds in the		on the Root Uptake of	
Troposphere	8	Radiocaesium	26
Organonitrogen and Sulphur Atmospheric		<b>5 Chemistry of the Geosphere</b>	<b>27</b>
Transformations	8	5.1 Geochemical Modelling	27
Acetone in the Atmosphere	9	5.2 Applied Geochemistry	28
Gasoline Additives	9	Methods	28
Atmospheric Chemistry of Aromatic		Terrestrial Geochemistry	28
Compounds	9	Marine Geochemistry	28
CEC KEENEX-Chemcom Project	10	5.3 Geochemical Technology	29
2.3 The Liquid Phase	11	Fixation of Heavy Metals in Soil	29
2.4 PAH and Field Atmospheric		Wet Oxidation of Organic Material	29
Chemistry	12	Marine Environmental Technology	29
2.5 Monitoring of Particulate		Corrosion Tests of Ceramics Materials	29
Fall-out During Exploratory		SANS-Measurements on Cementitious	
Activities at a Niobium		Materials	29
Mineralization in Greenland	13	Integral Experiments	30
<b>3 Plant Genetics and Biology</b>	<b>14</b>	5.4 General and Inorganic Chemical	
3.1 Barley Genetics	14	Analysis	30
Cytogenetics	14	5.5 Syntheses	31
Genetic Analyses of the Barley		5.6 MODECS	32
Genome	15	<b>6 Ecology and Mineral Cycling</b>	<b>33</b>
Quality of Barley Grain Protein	16	6.1 The MARINA Project	33
Barley Peroxidases	17	6.2 »SENSI«: A Model Describing	
Regeneration of Plants from Barley		the Accumulation and Time -	
Cells	17	Integration in the Bio-	
Embryogenic Barley Cell Suspension		Indicator Fucus Vesiculosus	
Cultures	17	of Radioactive Discharges	33
3.2 Resistance to Plant Diseases	18	6.3 Radioactive Contamination of	
Resistance to Powdery Mildew	18	Drinking Water	35
Biocontrol of Powdery Mildew	20	6.4 Fish Ecotoxicology	35
Resistance Mechanisms	20	6.5 Radioecological Cooperation with the	
Barley Leaf Stripe	21	USSR	36
Pathogen Genetics	21	<b>7 Other Activities</b>	<b>37</b>
3.3 Fungal Pathogens on Other		7.1 Deposition and Removal of	
Organisms	22	Radioactive Substances in	
<b>4 Nutrient Efficiency in Plant</b>		an Urban Area	37
Production	23	7.2 The Raman Group	38
4.1 Nitrogen in Soil and Plants	23	7.3 Dosimetry and Radiation Processing	38
Fate of N from Crop Residues and			
Manure	23		

<b>8 Large Facilities</b>	<b>39</b>
10 MeV Linear Electron Accelerator	39
Pilot Plant for Metal Extraction	39
Experimental Farm	39
Open Top Chamber Facility	40
<b>9 Publications</b>	<b>40</b>
9.1 Refereed Journals and Books	40
9.2 Proceedings (incl. Abstracts)	42
9.3 Research Reports	44
9.4 Other Publications	45
9.5 Presentations	45
<b>10 Education</b>	<b>51</b>
10.1 Ph.D. Projects	51
10.2 M.Sc. Projects	51
10.3 Contributions to Scientific Courses	51
10.4 University Lectures	51
<b>11 Exchange of Scientists</b>	<b>52</b>
<b>12 Cooperative Projects</b>	<b>52</b>
<b>13 Guest Lectures</b>	<b>54</b>
<b>14 Committee Memberships</b>	<b>55</b>
14.1 National	55
14.2 International	55
<b>15 Seminars Organized</b>	<b>56</b>
<b>16 Personnel</b>	<b>57</b>
16.1 Scientific Staff	57
16.2 Technical Staff	57
16.3 Office Staff	58
16.4 Ph.D. Students	58
16.5 M.Sc. Students	58
16.6 Apprentices	58
<b>17 Acronyms</b>	<b>59</b>

# 1 Introduction

This is the first annual report from the Environmental Science and Technology Department. The department was formed during the reorganization of Risø National Laboratory in March 1990, and includes the former Agricultural Research Department, part of the former Chemistry Department, and the Ecology Section from the earlier Ecology and Health Physics Department.

## Organization

The Environmental Science and Technology Department comprises approximately 123 scientific and technical staff members, and the department is organized in four sections with Dr. Arne Jensen as department head:

1. Ecology Section:  
head: Dr. Asker Aarkrog
2. Chemical Reactivity Section:  
head: Dr. Ole John Nielsen
3. Chemistry Section:  
head: Dr. Karen Nilsson (const)
4. Plant Biology Section:  
head of plant nutrition: Dr. Gunnar Gissel Nielsen.  
head of plant genetics and molecular biology: (vacant).

The curriculum of the department comprises research programmes over a wide range of subjects: atmospheric chemistry, chemical reactivity, geochemistry, geochemical modelling, hydrochemistry, analytical chemistry, organic synthesis, marine and terrestrial ecology, radioecology, trace metal ecology, plant nutrition, plant genetics, and plant molecular biology.

## Research objectives

Through basic and strategic research the department contributes to the solution of environmental problems related to energy production and consumption, industrial and agricultural production.

The aim of the department is to develop future technologies and methodologies for industrial

and agricultural production that will exert minimal stress on the environment.

The main research topics are:

The atmospheric environment  
Nutrient efficiency in plant production  
Plant genetics and biology  
Ecology and mineral cycling  
Waste treatment and recycling of industrial waste

Table 1 shows how these research topics are allocated and interrelated in the department, and how each section contributes to the departmental research effort.

## The Atmospheric Environment

The aim of the research programmes is to establish a sound scientific basis for rational legislative measures to reduce the industrial pollution of the atmosphere.

The research includes basic atmospheric chemistry, gas kinetics, transport and dispersion of air pollutants, especially nitrogen compounds, carbohydrates, PAH's, CFCs, HFCs and HCFCs. Atmospheric chemistry influences the climate, and the deposition of airborne pollutants can give rise to direct injuries to plants and indirect damage through bio-accumulation. To model and predict changes in the atmospheric composition, a better understanding of the chemical processes in the atmosphere is needed. In order to make the research results operative in environmental policy-making, the critical loads need to be established for natural and semi-natural ecosystems.

## Nutrient Efficiency in Plant Production

The aim of this research is to develop plant production techniques with effective assimilation and recycling of nutrients in order to reduce the probability for nutrient run off, and avoid losses of nutrients to the atmosphere and ground water.

The research emphasis is on biological, chemical, and physical processes involved in the turnover of plant nutrients, especially nitrogen and phosphorus. Further, biological nitrogen fixation and denitrification are emphasized together with

studies of the interaction between VA-mycorrhiza, plant roots and the rhizosphere.

### Plant Genetics and Biology

The research aim in this area is to develop crop plants with a reduced demand for nutrients and greater resistance to diseases and therefore needing less protection by use of pesticides. The growing of plants that are highly resistant to diseases and with less nutrient demand diminishes damage to the environment caused by high-yield plant production. Successful plant breeding research and biotechnology relies on attaining proficiency in a number of techniques such as cell and tissue culture, regeneration of plants, RFLP, RAPD, QTL, genetical analysis, protein chemistry, resistance biology and the ability to grow plants under controlled conditions.

### Ecology and Mineral Cycling

The aim of the research within ecology and mineral cycling is to improve the scientific basis of the studies, and attain a better understanding of the impacts of environmental pollution on marine and terrestrial ecosystems with emphasis on natural and semi-natural ecosystems. Another goal of the research is to establish an improved

knowledge base on the transport and fate of pollutants by use of radionuclides and trace metal techniques.

The research depends on concepts and techniques derived from radioecology. Concepts of environmental dosimetry and critical loads for ecosystems are developed further together with techniques for evaluating ecotoxicology in marine and terrestrial ecosystems.

### Waste Treatment and Recycling of Industrial Waste

In this area the department contributes to the scientific basis for developing the wet oxidation technique and new methods for conditioning and treating polluted soil, sludge, sewage and other waste products, including radioactive waste, from industrial operations.

The aim of the research programme is to model the migration of heavy metals and nutrients, and predict the fate of deposits in geological structures and soils. Existing toxic waste deposits have created severe environmental problems in industrialized countries, and the department plans to develop methods to repress the uncontrolled release of toxic material from waste deposits.

*Table 1.1. Department of Environmental Science and Technology research topics*

Section	Atmospheric environment	Nutrient efficiency in plant production	Plant genetics & biology	Ecology and mineral cycling	Waste use & treatment	Other activities
Chemistry	Nitrogen compounds			Marine environment	Geochemical modelling	Chemical analysis
	Transport and dispersal Atmospheric chemistry methods				Technology for waste treatment	Synthesis MODECS
Chemical Reactivity	Nitrogen compounds Basic atmospheric chemistry					Accelerator  Resonance Raman
Plant Biology	Nitrogen compounds	Turnover of nutrients  Biological interaction in soils Denitrification	Barley genetics Resistance biology Protein quality Fungal inhibiting proteins Fungal virulence Cell culture	Terrestrial environment	Technology for waste treatment	Dyskørgård (the experimental farm)
Ecology	Nitrogen compounds  Contamination physics			Marine environment Terrestrial environment Environmental dosimetry		Radiological control & preparedness



## 2 The Atmospheric Environment

### 2.1 Introduction

During the last decade the effect of human activities on the global atmosphere has become increasingly evident. Observations of phenomena such as the «Antarctic Ozone Hole», «Acid Deposition», «Greenhouse Effect» and «Urban Smog» have led to increasing public concern about the global impact of human made emissions on the chemistry of the atmosphere. The key to understanding these phenomena and predicting future effects is the ability to accurately model the chemistry of the atmosphere. The basis for this modelling is the use of kinetic and mechanistic data obtained in laboratory studies concerning atmospheric reactions of primary and secondary pollutants and their products.

Part of the department's atmospheric research is concerned with chemical kinetics in the gas and liquid phase and takes place in the Section for Chemical Reactivity. One important part of this work is our continued laboratory effort to investigate the kinetics and mechanisms of the

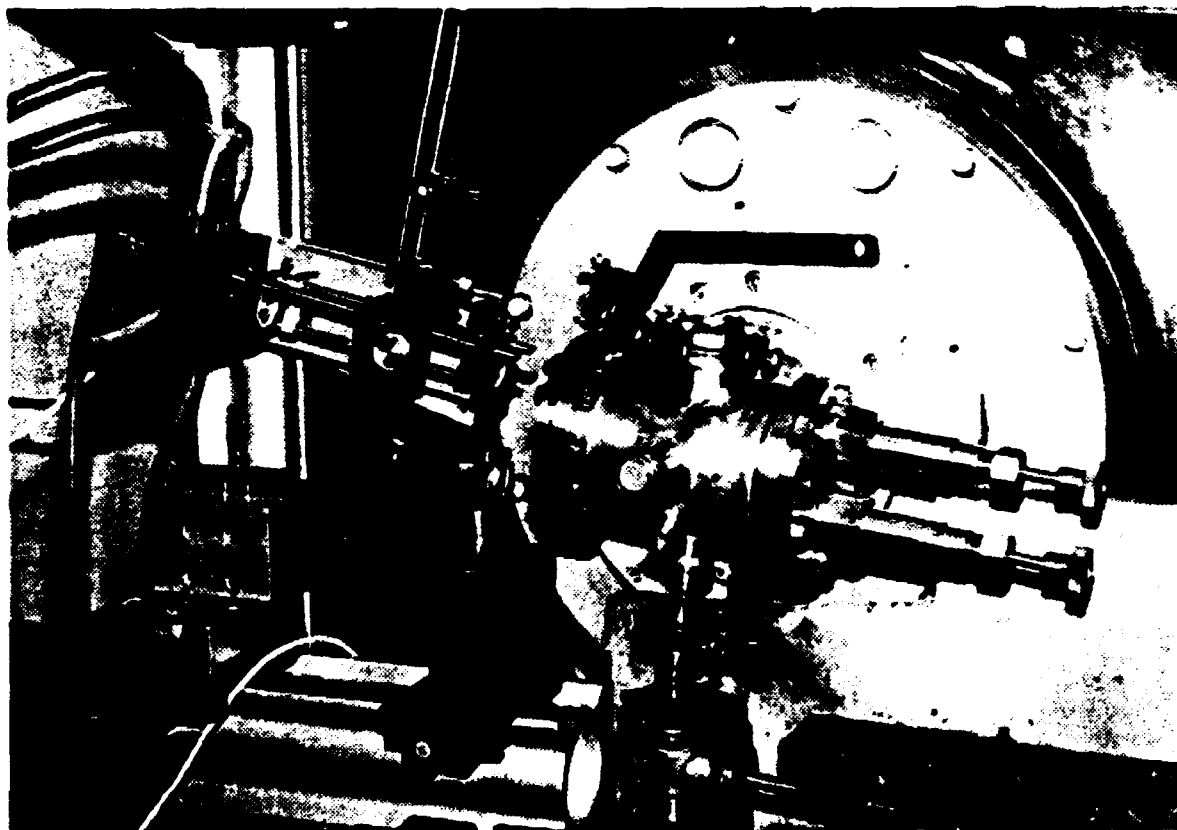
reaction of OH radicals with a variety of hydrocarbons and substituted hydrocarbons. This work on OH radical kinetics was part of several projects in 1990.

### 2.2 The Gas Phase

#### A New Group of Chemicals

The international concern about the effects of depleting stratospheric ozone has forced urgent consideration of possible alternative substances to replace the currently used fully halogenated chlorofluorocarbons (CFCs) responsible for the depletion. The general task is now to determine the environmental acceptability of the alternatives. In order to evaluate their potential effects it is necessary to know the atmospheric chemistry of the alternative compounds, to know all relevant rate constants at all relevant pressures and temperatures, the primary reaction products and their further reactions. Even for a small number of potential substitutes this is an enormous task.

*Figure 2.2.1. The field emission accelerator in the gas phase kinetic experimental setup.*



Therefore, it is necessary to create a predictive database. Alternative compounds considered or in use at the moment are hydrofluorocarbons (HFCs) containing only carbon, fluorine and hydrogen atoms and hydrochlorofluorocarbons (HCFCs) containing carbon, fluorine, chlorine and hydrogen.

Because these compounds contain hydrogen they react with hydroxyl radicals, OH, in the troposphere and hence have shorter atmospheric lifetimes than the fully halogenated CFCs. As a consequence, they have less chance of reaching the stratosphere intact. However, this does not eliminate the compounds from contributing to stratospheric O<sub>2</sub> destruction or other environmental effects. We have clearly demonstrated this in a recently published study of the atmospheric oxidation of methylchloroform, CH<sub>3</sub>CCl<sub>3</sub>, performed in collaboration with University College Dublin. We concluded that it is necessary to study the full atmospheric cycle of the degradation products in order to be able to evaluate the potential environmental effects of these compounds.

As part of the Danish Environmental Protection Agency CFC research programme we have measured the rate constant for reactions between OH and a series of HCFCs at different temperatures, and calculated their atmospheric lifetimes. When OH reacts with an HCFC molecule, a halogenated alkyl radical is formed, which will react with atmospheric oxygen. Investigations of this next step in the atmospheric degradation pathway are being pursued.

### Halogenated Organic Compounds in the Troposphere

This is a STEP project involving 9 European laboratories concerned with the kinetics and mechanisms of oxidation of chlorine and bromine containing compounds under atmospheric conditions. It is the aim of this study to determine the tropospheric sinks for these halogenated compounds in order to provide the basis for calculating the ozone depletion potential for released halogenated species. The part of this work concerned with the spectra and kinetics of peroxy radicals is being performed in collaboration with Dr. T. Wallington, Ford Motor Co., USA. The first result of this work on the peroxy radicals of HCFC-141a and HCFC-142b has been accepted for publication.

### Organonitrogen and Sulphur Atmospheric Transformations

1990 was the third year of this four-year CEC-funded collaboration with University College, Dublin. We have investigated the reactions of Cl atoms and OH radicals with a wide variety of compounds: alcohols, ethers, dialkylsulphides, nitroalkanes, n-alkyl nitrates and n-alkyl nitrites. The part of this work that dealt with the reaction of OH radicals with nitroalkanes at low pressure and different temperatures was performed at National Institute of Standards and Technology, USA, in collaboration with Dr. M. Kurylo.

Rate constant data from pulse radiolysis and relative rate experiments for the reaction of the OH radical with n-alkyl nitrites at 1 atm total pressure are in good agreement. The rate constant for the reaction with methyl nitrite is close to that previously reported for the OH + C<sub>2</sub>H<sub>6</sub> reaction. This suggests that replacing a methyl group by a nitrite group in ethane has very little effect on the reactivity with OH radicals. This result is somewhat unexpected since if this reaction involved only H atom abstraction the presence of the electron withdrawing ONO group would be expected to considerably decrease the rate constant for abstraction by the electrophilic OH radical. The rate constant for the reaction of Cl atoms with CH<sub>3</sub>ONO determined in this work shows a decrease of about a factor of 3 compared to the value for the Cl + C<sub>2</sub>H<sub>6</sub> reaction. The reaction of the electrophilic Cl atoms with substituted organics is believed to involve a hydrogen atom abstraction process, hence the lack of any reduction in the reactivity for the corresponding OH radical reaction indicates that there may be some mechanistic difference between the two systems.

The reaction between OH radicals and methyl nitrite may involve both hydrogen abstraction and OH addition followed by rapid decomposition of the adduct. From the above kinetic evidence it is proposed that the reaction of OH radicals with CH<sub>3</sub>ONO may have an addition component at atmospheric pressure.

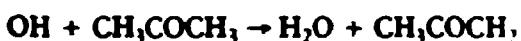
The rate data determined in this work shows that the major loss process in the atmosphere for alkyl nitrites is photodecomposition. In daylight nitrite rapidly photolyse back to the original reactants. Thus alkyl nitrites serve only as a temporary NO<sub>x</sub> reservoir for alkoxy radicals and NO during the night. Recently, methyl nitrite has been detected in the exhaust from methanol

burning engines, indicating that alcohol-based fuels will contribute to alko radical formation during daylight hours.

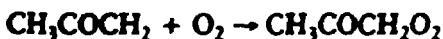
Reactions of OH radicals with n-alkyl nitrates have been shown to involve both hydrogen atom abstraction and addition followed by rapid decomposition of the adduct. Previously reported data from our laboratories suggested that for  $\text{CH}_3\text{ONO}_2$  the addition channel is the main reaction process at 1 atm and 298 K while for longer chain compounds abstraction is dominant. The results obtained in the present study support this view. A small negative activation energy of  $-2.1 \text{ kcal mol}^{-1}$  was obtained for the  $\text{OH} + \text{CH}_3\text{ONO}_2$  reaction. The activation energy for  $\text{OH} + \text{C}_2\text{H}_5\text{ONO}_2$  is somewhat less negative:  $E_a = -1.4 \text{ kcal mol}^{-1}$ , and that of n-propyl nitrate is close to zero. Those results suggest that at least for methyl and ethyl nitrate addition is the dominant pathway at atmospheric pressure. As the length of the side chain increases this pathway becomes less important as the deactivating effect of the nitrate group on the abstraction process becomes less significant.

#### Acetone in the Atmosphere

Acetone is a widespread trace constituent of the atmosphere. It originates as a product from the oxidation in the atmosphere of a range of hydrocarbon species, as well as from direct emission from its use as a solvent. In the lower troposphere the lifetime of acetone is controlled mainly by the reaction with OH to produce water and the acetylonyl radical:



In the atmosphere the acetylonyl radical reacts with  $\text{O}_2$  to give the acetylonylperoxy radical:



We have recorded the ultraviolet absorption spectra of these two radicals and studied the kinetics of their reactions. The work was done in collaboration with Dr. R.A. Cox from Harwell Laboratories, UK (Chem. Phys. Lett. v.173 (1990) p. 206).

#### Gasoline Additives

Tetraalkyllead compounds have been added to gasoline to prevent pre-ignition since 1923 when

tetramethyllead and tetraethyllead were first used in the United States. Most of the organic lead compounds are decomposed during the combustion process and emitted as a complex mixture of lead salts, although incomplete combustion, spillage and evaporation results in about 2% of the alkyllead content of fuel entering the atmosphere being unchanged. For several decades the use of tetraalkyllead additives has given rise to environmental concern and has led to legislation to limit their use. However, they are still extensively employed in many countries, and the rate and mechanism for their atmospheric degradation are of some importance.

It can be concluded that the main atmospheric removal pathway was initiated by reactions with OH radicals and that removal by photolysis of reaction with  $\text{O}_3$  and  $\text{O}(^3\text{P})$  atoms was of less importance. Rate constant data for the reactions of OH with tetramethyl and tetraethyllead have been determined by several investigators including ourselves. Although the reported rate constants for  $(\text{CH}_3)_4\text{Pb}$  are in reasonable agreement, those for  $(\text{C}_2\text{H}_5)_4\text{Pb}$  disagree by a factor of about 7.

The aim of this work was to redetermine rate constants at atmospheric pressure and room temperature for the reactions of OH radicals with tetramethyl and tetraethyllead using both the absolute technique of pulse radiolysis combined with UV kinetic spectroscopy and a conventional relative rate method. It was hoped that the results would resolve the discrepancy in the previously reported rate constants for these reactions and provide reliable data in order to evaluate the atmospheric lifetimes of these species. The reactions of OH radicals with the analogous carbon compounds  $(\text{CH}_3)_4\text{C}$  and  $(\text{C}_2\text{H}_5)_4\text{C}$  were also investigated with a view to providing further information on the reactivity trends for these reactions.

This work was done in collaboration with Dr. H. Sidebottom, University College Dublin and has now been accepted for publication. The results using two different techniques were in agreement within the experimental uncertainties and the previous discrepancies now seem to have been solved.

#### Atmospheric Chemistry of Aromatic Compounds

We are engaged in a joint European research project on aromatic compounds within the CEC

## STEP PROGRAMME.

The degradation of aromatic compounds in the atmosphere is initiated by attack of hydroxyl radicals which leads to the formation of adducts, e.g. in the case of benzene:



Recent laboratory studies indicate that the fate of the adduct is mainly controlled by the reaction with  $\text{O}_2$ . However, the reaction of the adduct with  $\text{NO}_2$  is also thought to be of some importance in the polluted troposphere.

The aim of the experimental work carried out at Risø is to record ultraviolet spectra of the short-lived OH-adducts of benzene and other aromatic compounds. Next we want to study the chemical reactivity of the various OH-adducts, e.g. the reactions with  $\text{O}_2$  and  $\text{NO}_2$ .

We have employed a series of different source reactions for hydroxyl radicals. By pulse radiolysis of  $\text{Ar}/\text{H}_2\text{O}$  mixtures the hydroxyl radicals are produced by the reaction of metastable Ar-atoms with water molecules:



In the presence of small amounts of benzene we observe transient absorption signals around 300 nm. The transient absorption was tentatively assigned to the adduct  $\text{HO-C}_6\text{H}_6$  produced via reaction (1). However, it seemed likely that the  $\text{H-C}_6\text{H}_6$  adduct produced via reaction (2) might also contribute to the observed transient absorption signals:



The UV-spectrum of the H-atom adduct was obtained by pulse radiolysis of  $\text{H}_2/\text{C}_6\text{H}_6$  mixtures and it was found that the UV-spectra of  $\text{H-C}_6\text{H}_6$  and  $\text{HO-C}_6\text{H}_6$  are very similar in shape.

Thus, the transient absorption signals observed by pulse radiolysis of  $\text{Ar}/\text{H}_2\text{O}/\text{C}_6\text{H}_6$  mixtures are composed of contributions from both types of radicals.

To overcome these difficulties we have used the OH source reaction (3) which was initiated by pulse radiolysis of  $\text{SF}_6/\text{H}_2\text{O}$  mixtures:



In the presence of small amounts of benzene we expect to observe the formation of  $\text{HO-C}_6\text{H}_6$

in accordance with reaction (1). However, it was found that the competing reaction (4) is important even at high  $\text{H}_2\text{O}/\text{C}_6\text{H}_6$  concentration ratios:



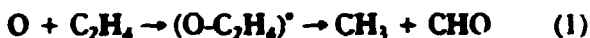
The UV-spectrum and kinetics of the self-reaction of  $\text{F-C}_6\text{H}_6$  were studied by pulse radiolysis of  $\text{SF}_6/\text{C}_6\text{H}_6$  mixtures. The F-atom adduct has a very strong absorption band around 300 nm and the absorption cross section is at least an order of magnitude greater than that of the OH-adduct. The spectral overlap as well as the experimental evidence that the ratio of the rate constants  $k_4/k_1 = 20$  have made it extremely difficult to obtain the UV-spectrum of  $\text{HO-C}_6\text{H}_6$  without contributions from the F-atom adduct. However, UV-spectra obtained with a very high  $\text{H}_2\text{O}/\text{C}_6\text{H}_6$  concentration ratio can certainly be assigned to the OH-adduct.

## CEC KEENEX- Chemcom Project

This CEC JOULE project on combustion kinetics is coordinated by HARWELL, and the experimental studies at Risø are carried out in close collaboration with Christopher Anastasi at the University of York and Anders Lund at the University in Linköping, Sweden.

The main task of the Chemcom Project is to obtain fundamental experimental data on the elementary reactions involved in combustion processes, particularly those reactions affecting efficiency and pollutant formation. The Chemcom Project started in August 1990, and it will last 30 months.

The experimental studies at Risø are carried out using pulse radiolysis combined with time-resolved UV- and IR-spectroscopy. Since the start of the programme we have been studying the chemical reaction mechanisms involved in the oxidation of alkenes initiated by the reactions with O-atoms and OH-radicals. In the case of ethylene we have observed the reactions (1) and (2).



The mechanism of reaction (1) is quite complex and we have studied the kinetics by monitoring the formation and decay of methyl radicals. The reaction has been investigated over a

wide range of pressures using IR-spectroscopy in the low-pressure regime, 10-100 mbar and UV-spectroscopy at higher pressures, 100-1000 mbar where IR-spectroscopy could not be used because of pressure broadening of the vibration-rotation lines of CH<sub>3</sub>.

The kinetics of reaction (2) has been studied and the UV-spectrum of HO-C<sub>2</sub>H<sub>4</sub> has been recorded. Based on this spectrum we have been able to study the kinetics of reaction (3).

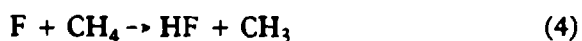


The peroxy radical produced in reaction (3) has an ultraviolet absorption band with a maximum at 230 nm. Work is in progress to study the kinetics and mechanism of the self-reaction of the peroxy radical.

Part of the Risø research programme is devoted to examining reactions between different types of free radicals. The experimental technique employed at Risø is more useful than most other applied techniques because of the very high radical yields obtained by pulse radiolysis.

The reactions (4) - (8) were initiated by pulse radiolysis of SF<sub>6</sub>/CH<sub>4</sub>/H<sub>2</sub>O mixtures.

The F-atoms produced initially are converted into a mixture of methyl and hydroxyl radicals with a concentration ratio controlled by the relative amount of CH<sub>4</sub> and H<sub>2</sub>O added to the gas mixture.



The transient absorption signals of CH<sub>3</sub> and OH were monitored at 216.4 and 309 nm, respectively. The rate constant for the cross combination reaction (7) was evaluated by computer simulations of the experimental decay curves.

## 2.3 The Liquid Phase

The investigation of the ozone decomposition in the aqueous acidic solution initiated by H<sub>2</sub>O<sub>2</sub> was continued. The results show that the thermally initiated O<sub>3</sub> decomposition is a heterogeneous

reaction which depends on the surface and volume of the reaction vessel. The initial rate of O<sub>3</sub> decomposition is measured as a function of concentrations of both H<sub>2</sub>O<sub>2</sub> and O<sub>3</sub> in vessels with different surface-to-volume ratios. In some experiments acetic acid was employed as OH-scavenger in an attempt to retard the chain decomposition. The results from these experiments also indicate a surface initiation of the thermal O<sub>3</sub> decomposition. Indications of surface termination of the chain reaction are obtained in experiments with low concentrations of H<sub>2</sub>O<sub>2</sub>.

The rates of the oxidation reactions of ferrous compounds with ozone were measured in acidic solution by a stopped-flow technique. An intermediate complex, likely to be the ferryl ion, lasting for tens of seconds was discovered. Rate constants of the complex reacting with Fe<sup>++</sup>, O<sub>3</sub> and H<sub>2</sub>O<sub>2</sub> were determined and a spectrum of the complex has been recorded. From stoichiometric measurements and the rate of disappearance of the complex, a mechanism involving the release of an OH radical may be inferred. The Fe-O<sub>3</sub> complex reacts differently in HClO<sub>4</sub> and H<sub>2</sub>SO<sub>4</sub> probably because of complexation with sulphate ions in H<sub>2</sub>SO<sub>4</sub>.

Peroxy radicals play an important role in the oxidation of hydrocarbons in the atmosphere. A project on the oxidation of methane has been initiated by studying the peroxymethyl radical in aqueous solution under high pressure of methane by pulse radiolysis.

The work on high-temperature and high-pressure aqueous radical chemistry has been continued by studying the reactions: e<sub>aq</sub><sup>-</sup> + OH, e<sub>aq</sub><sup>-</sup> + H<sub>2</sub>O<sub>2</sub> and Fe<sup>++</sup> + H<sub>2</sub>O<sub>2</sub> including thermal degradation of H<sub>2</sub>O<sub>2</sub>, determining the rates and activation energies. Some computer simulations on the reaction mechanisms at high temperature have been performed as well.

The reactivity of the primary water radicals towards pyridine derivatives has been measured. Rate constants and absorption spectra of radicals of several carboxy- and methyl-substituted pyridines have been determined. These investigations are carried out in collaboration with the University of Vienna.

Sulphate radical anion, SO<sub>4</sub><sup>-</sup> is an important species in atmospheric aqueous oxidation of S(IV). Reactions of the sulphate radical with other relevant radicals like OH, HO<sub>2</sub>, O<sub>2</sub><sup>-</sup> have been studied by pulse radiolysis. Computer simulations necessary to obtain the corresponding rate constants are in progress.

Investigations have been continued on intramolecular electron transfer in small peptides between tryptophan and tyrosine units that are separated by different spacer sequences. The results give further evidence against the existence of the postulated through-bond electron transfer.

Mechanisms of OH-radical-induced decarboxylation of small methionine-containing peptides,  $\gamma$ -glutamylmethionine and S-alkylglutathione derivatives have been investigated in cooperation with the Polish Academy of Sciences and Hahn Meitner Institute in Berlin.

## 2.4 PAH and Field Atmospheric Chemistry

Air pollution by PAH (polycyclic aromatic hydrocarbons) has been investigated for many years, one reason being that several PAH are considered to be carcinogenic. The atmospheric chemistry of PAH has been studied in detail. Analytical methods have been developed as well as methods for determining the source contributions.

An investigation into the presence of PAH at and in the vicinity of the international airport of Copenhagen was completed in 1990. The investigation included vapour phase PAH as well as particle-adsorbed PAH. The amount of air pollution was estimated at two locations and compared with that at other locations. The annual average of benzo(a)pyrene at the airport is estimated to be in the magnitude of  $2 \text{ ng/m}^3$ . The sources are motor traffic and combustion, including that from long-distance air transport. The total contribution from surface traffic at the airport and that from air traffic are estimated to be  $15 \pm 20\%$  with surface traffic dominant 80-85% from the city. The composition of the PAH varies with the composition of the sources. A considerable part of the lighter PAH, e.g. phenanthrene, is present in vapour phase. The distribution of the lighter PAH between vapour phase and particulates depends on the ambient temperature and concentration of soot particles. Only a small part of the carcinogenic PAH is in vapour phase. The air pollution of PAH correlates with the air pollution of mutagenic compounds, particles,  $\text{NO}$ ,  $\text{NO}_x$ ,  $\text{CO}$ ,  $\text{SO}_2$  and hydrocarbons.

The extent of air pollution was relatively minor at the airport compared with that of busy streets in the inner city of Copenhagen. Sources of PAH were automobiles, heating systems and long-range transport, whereas the contribution

from aircraft turned out to be small. Three new projects have been planned and organized in 1990. These are:

- a) Within DCAR (Danish Center of Atmospheric Research) a traffic PAH project providing PAH data from different locations in the initial phase of the introduction of catalyst-equipped cars in order to be able to follow the effect. The project also aims at determining the contributions of gasoline driven cars and diesel-cars, respectively.
- b) Within EUROTRAC, a DCAR-TOR (Tropospheric Ozone Research) project. The aim is to determine the budget of the strongly increasing ozone, the seasonal variations, and the factors influencing the ozone level.
- c) Applying and improving the DOAS (Differential Optical Absorption Spectroscopy) technique for determinations of air pollutants. The DOAS technique can be used for determining several air pollutants, e.g. it is one of the few techniques being applicable for continuous specific registration of  $\text{NO}_2$ .

Figure 2.4.1. Jens Østergaard Larsen and Torben Nielsen taking samples at the airport in Copenhagen.



Besides, it is the only known technique that is applicable for determining  $\text{NO}_3$ . The  $\text{NO}_3$  radical is extremely important for its contribution to effect on the night time chemistry of the atmosphere.

## 2.5 Monitoring of Particulate Fall-out During Exploratory Activities at a Niobium Mineralization in Greenland

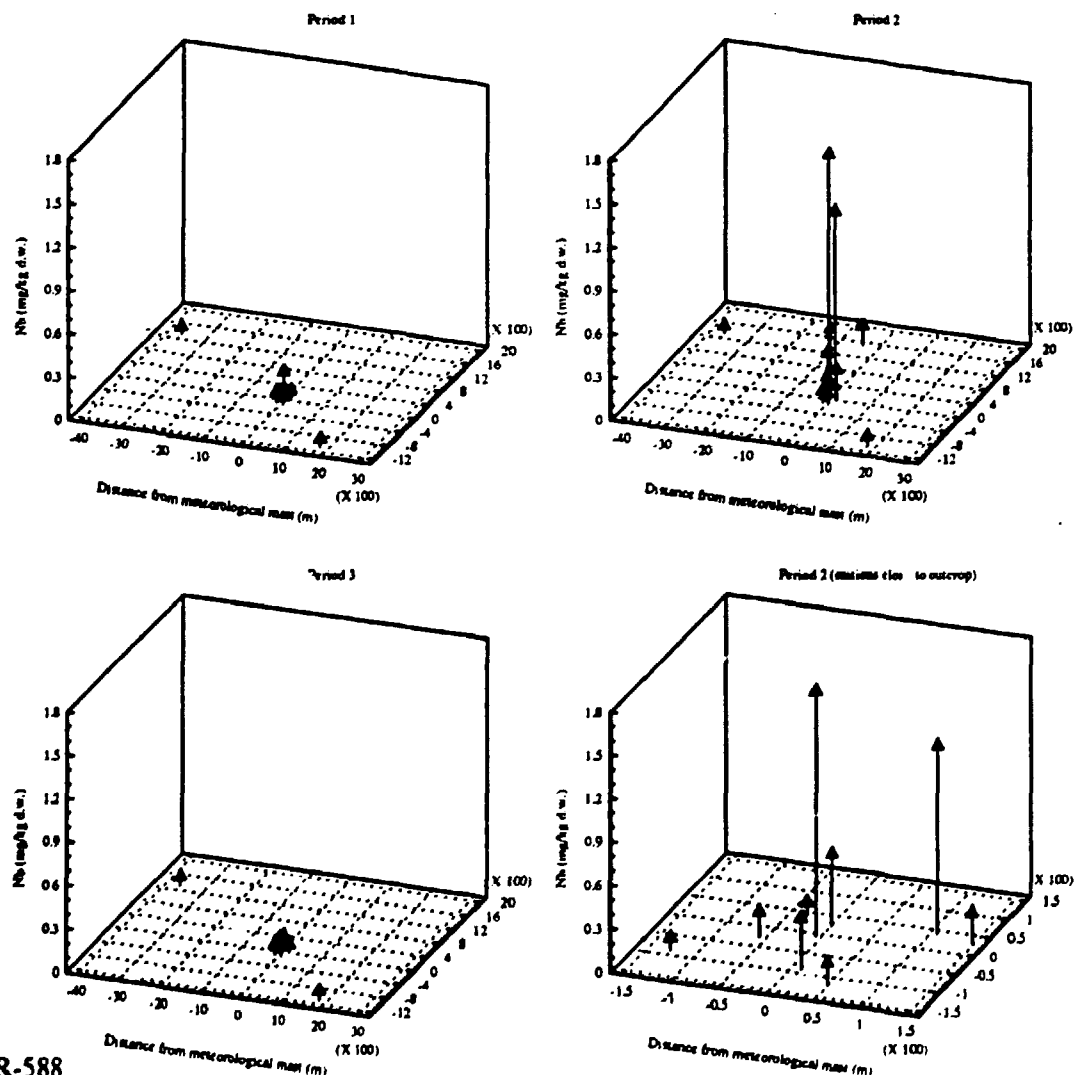
The main purpose of the investigation was to monitor airborne dispersion of particulates in connection with exploration activities (drilling, trenching, blasting) at the Nb-mineralization in the Sarfartoq area, SW Greenland.

Due to the lack of logistic facilities in the Sarfartoq area the possibilities of operating conventional dust monitors are poor. Thus, it is not possible to operate dust monitors in the form of filters with electric pumps. The only realistic

possibility is to use passive dust monitors. Therefore, the biological monitoring technique was used in the form of moss bags and samples of indigenous mosses and lichens. Following this a secondary purpose of the investigation was to evaluate the applicability of this technique on location.

Moss bags (comprised of *Sphagnum girgensohnii* from a background area) were placed close to nine test sites previously laid out by the Environmental Survey of Greenland in the area around the ore region in Sarfartoq. In addition, moss bags were placed at three background sites. Three sets of bags were exposed: one set before major dust-producing activities, another during the period of drilling, and a third after the work was completed. Samples of the indigenous vegetation (the lichen *Cetraria nivalis* and the moss *Hylocomium splendens*) were taken before and after the activity. The moss bags and other samples were analyzed for the elements Zn, Nb, Cd, LREE (light rare earth elements), Pb, Th and U

Figure 2.5.1 Niobium ( $\mu\text{g/g d.w.}$  in moss bags).



by ICP-MS (Inductively Coupled Plasma Mass Spectrometry). Nb, LREE, Th and U are enriched in the mineralization; Cd and Pb are included because they are toxic and common pollutants.

The analyses of element concentrations in the indigenous vegetation showed that the natural pollution (*i.e.* the situation prior to any human dust-producing activities) in the area is restricted to areas immediately at or on the outcrop. The elements found in elevated concentrations are Nb, La, Ce, Th and U. These elements are highly enriched in the Nb-mineralization.

The monitor organisms show a rise in the concentrations of some of these elements following

the exploration activities during the summer. This shows that some dust has been dispersed in the area. The extension of the pollution is however restricted to stations very close to the outcrop, and the magnitude is low. This is in accordance with the kind of exploration work carried out (trenching and drilling) which does not produce substantial amounts of dust.

The use of moss bags can be recommended for future investigations because they are easily related to dust-producing activities, and *Cetraria* can be recommended because it reacts effectively to a change in aerial deposition. In order to keep the methodological variation low, replicates should be used at all moss bag stations.

### 3 Plant Genetics and Biology

#### Plant Genetics

The genetic variation in cultivated plants is extended, shaped and utilized by plant breeding to produce new crop plant varieties with high yield, good quality, and resistance to plant diseases. The quality characteristics may be agronomical, technological or nutritional. Disease resistance adds to a high, stable yield and reduced use of pesticides. A basic requirement for efficient plant breeding is knowledge about the genes that govern the desired traits, their interaction with other genes, the location of the genes on the chromosomes and genetic recombination. Our aim is to provide knowledge on the genetics and biology of crop plants, to develop and refine conventional and molecular biological techniques applicable to plant breeding, and to produce germ plasm with genes for the desired traits.

#### 3.1 Barley Genetics

The work in 1990 has brought substantial progress in the application of new technologies to our main topics: the genetic and molecular analyses of the barley genome, particularly quality traits and disease resistance.

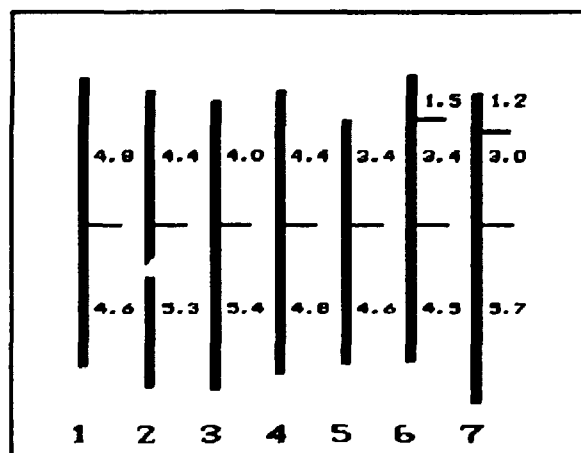
#### Cytogenetics

Giemsa C- and N-banding techniques have made possible an unequivocal identification of the seven barley chromosomes and their arms. As a consequence, it is now possible to estimate reliably the length of the individual chromosomes

and chromosome arms. Barley chromosomes identified by banding were measured by use of a digitizer. Both actual and relative chromosome lengths as well as distances between individual chromosomal markers were non-normally distributed and had to be logarithmically transformed. The back-transformed lengths of the chromosomes are given on the idiogram presented in Fig. 3.1.1. The 95 per cent confidence limits of the mean lengths are approximately  $\pm 2$  per cent of the lengths. Chromosome 1 showed arm length reversal in 35 of the 100 cells used. In the other six chromosomes, this reversal occurred at a number of various frequencies, all lower. Thus, the chromosomes and their arms in a single cell cannot be identified unambiguously by length.

The traditional use of the designations »short«

Fig. 3.1.1. Idiogram of the seven chromosomes of barley.





and «long» chromosome arms suggests that chromosome arms can be identified by their length. This is obviously not correct. Therefore, we have proposed that:

- 1) One chromosome arm with a specific banding pattern is designated the «plus» arm and is drawn upwards in the idiogram; the other arm is designated the «minus» arm;
- 2) Total genome length is defined as one «GeNome» (symbol GN);
- 3) A distance on the idiogram is given in milli-GeNomes (symbol mGN);
- 4) The centromere is assigned the position 0;
- 5) Cytological markers on the «plus» and «minus» arms are designated by their positive and negative distances (in mGN) from the centromere (position 0), respectively, i.e. distances between markers are obtained by subtraction.

This system for designating arms and positions of cytological markers on the barley chromosomes is illustrated in Fig. 3.1.2. The system should be applicable to all species in which the individual chromosome arms can be identified.

In collaboration with the Institute for Plant Breeding, Quedlinburg, Germany, Giemsa C-banding was used to identify single *Hordeum chilense* chromosomes in a material of *Secale cereale*-*H. chilense* addition lines with  $2n = 15$ . Six of the seven *H. chilense* chromosomes were found as individual additions. *H. chilense* chromosome 1 was present only in one plant with  $2n = 20$  having six *H. chilense* chromosomes.

Giemsa C-banding has further been used to study the species relationship of the two species of the genus *Dasyphyrum*, viz. the diploid *D. villosum* and the tetraploid *D. breviaristatum* (syn. *D. hordeaceum*). Both contain genes of interest for plant improvement. Some evidence has pointed to *D. breviaristatum* as an autotetraploid derivative of *D. villosum*. In a Greek material provided by Dr. Signe Frederiksen, Botanical Laboratory, University of Copenhagen, the Giemsa C-banded karyotypes differed significantly both with respect to banding patterns and marker chromosome morphology challenging the role of *D. villosum* as the sole progenitor of *D. breviaristatum*.

The plant *Miscanthus sinensis* «Giganteus» has recently attracted attention as a source of organic dry matter and fibre production in northern Europe. A cytological study showed that it is a triploid with  $2n = 57$ . This and the presence of

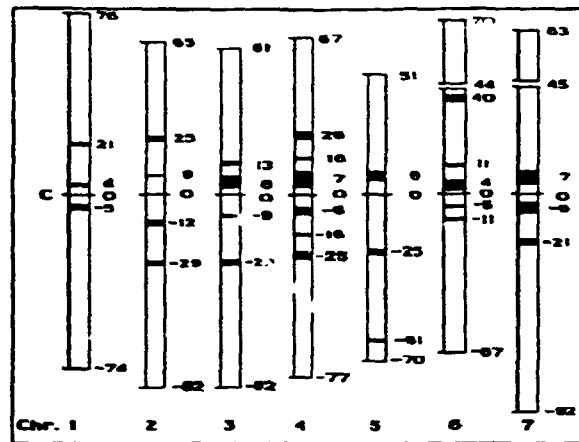


Fig. 3.1.2. Idiogram of the Giemsa C-banded chromosomes of barley. Positions of cytological markers in mGN (milliGeNomes).

only one chromosome with a silver nitrate stained nucleolus organizer region indicate that *M. sinensis* «Giganteus» is an interspecific hybrid with one parent having provided two and the other parent one genome.

### Genetic Analyses of the Barley Genome

Barley RFLP (Restriction Fragment Length Polymorphism) markers are now routinely developed and 21 have been used in linkage mapping. Seventy-two chromosome-doubled haploid spring barley lines from a cross between a winter and a spring barley variety were used in the analysis. The 14 marker gene loci with known chromosomal location: *v*, *Mla6*, *Mlh*, *MI(La)*, *Hor1*, *Hor2*, *Prx4*, *Pgd2*, *Amy1*, *Est4*, *Est5*, *Est9*, *Acp2*, and *Aco1* were included. Three linkage groups including QTLs (Quantitative Trait Loci) for grain yield could be assigned to specific chromosomes. One is illustrated in Fig. 3.1.3. This linkage map contains two groups of linked loci combined by a common QTL for grain yield on chromosome 6. As the standard deviation is about 10 cM, the order of the loci is preliminary.

Contact has been made with international groups also working on genetic analyses of barley using RFLP markers. A set of 50 lines including spring and winter forms from our cross will be selected for general mapping purposes. In collaboration with Danish plant breeders two new crosses between modern spring barley varieties and one cross between winter barley varieties are being developed from parents selected for maximum variation in hordein and isozyme patterns. Chromosome-doubled haploid lines from

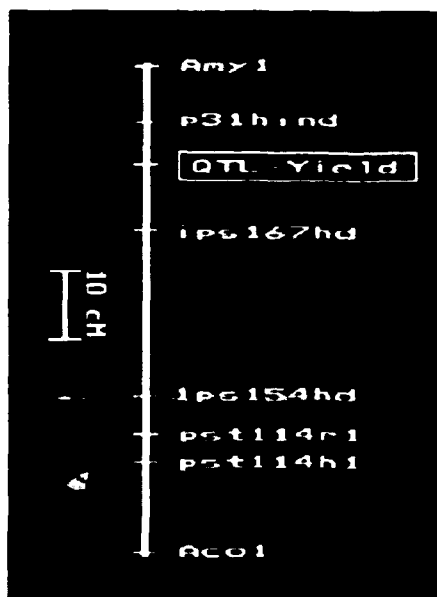


Fig. 3.1.3. A linkage group of marker genes, RFLP loci, and a QTL for grain yield on barley chromosome 6.

these crosses will be the basis for our long term mapping efforts and analyses of QTLs.

A study has been initiated to test the potential for using RFLPs in accelerating backcross breeding. Backcross progeny from a cross between the variety »Corgi« and two Ethiopian barley lines carrying genetically unidentified powdery mildew resistance genes, has been selfed, and further backcrossed to »Corgi«. DNA has been extracted from the backcross lines and is being tested with RFLP markers to select lines that mostly resemble the Ethiopian line and »Corgi«, respectively. A population of offspring from second backcross lines derived from these plants will be analysed by a new set of RFLP markers. Variation in the backcross population will be determined in the selfed lines.

One issue of considerable concern to geneticists and plant breeders alike is whether disease resistance genes have adverse effects when the disease is absent. A recently completed study did not disclose any negative effects on important agronomic properties in disease-free environments of 17 race-specific powdery mildew resistance genes in at least five loci in barley. QTLs negatively affecting agronomic traits were not detected in any of the cases where race-specific powdery mildew resistance genes have been transferred by backcrossing to high-yielding barley varieties. An exception was the resistance genes in locus *mlo*. They reduce grain yield,

mainly through reduced grain size, by about 4 per cent on average, but irrespective of the presence or absence of powdery mildew. This is due to necrotic leaf spotting, a wellknown pleiotropic effect of the *mlo* genes. The necroses and subsequent early senescence of the barley leaves reduce the green leaf area, and the translocation of photosynthetic products from leaves to grains. This undesirable effect of *Mlo* resistance can, however, be eliminated by plant breeding. The conclusion is that powdery mildew resistance genes in barley have no harmful effects in disease-free environments.

### Quality of Barley Grain Protein

The storage protein of the barley grain (hordein) is poor in the essential aminoacid lysine. The nutritional quality of barley may be improved by an increased synthesis of proteins rich in lysine. Protein Z is a barley seed storage protein with a favourable lysine content. It is coded for by genes in two loci, one on chromosome 4, *Paz4* (protein Z4), and the other on chromosome 7, *Paz7* (protein Z7). Our aim is to increase the amount of protein Z by two different approaches. In one we try to duplicate locus *Paz4* by classical genetic methods. In the other we isolate protein Z genes and attempt to increase the number of gene copies by genetic transformation. Both procedures imply that the increased number of gene copies will lead to an increase in protein Z production as well as lysine content in the barley grain.

Locus *Paz4* is the target for duplication using reciprocal translocations to duplicate a short chromosome segment carrying this locus. One hundred crosses have been made between barley lines carrying translocations involving chromosome 4. The  $F_2$  seeds are being tested for the amount present of protein Z, using antibodies raised towards purified protein Z4. Theoretically, among 16  $F_2$  seeds one should lack protein Z4, and one should carry a duplication with locus *Paz4*. An analysis of the crosses has not revealed any no-Z4 seeds, but one cross showed a high degree of variation in the amount of protein Z4. This material is kept for analyses of future generations. Another cross showed a change in the proportion between proteins Z4 and Z7, which may suggest the presence of a duplication.

Two genomic DNA clones and one cDNA clone encoding three different proteins each similar to protein Z4 have been isolated. The 3286 nucleotide long sequence of one genomic clone

has been established, and the deduced amino acid sequence shows approximately 85% sequence identity to that determined for purified protein Z4. Preliminary sequence data for the two other clones also show a high sequence identity to protein Z4. This could indicate that *Paz4* is a complex locus with several closely related genes. Both nucleotide sequences contain an intron in the same position in the coding region, but the lengths of the introns are different; the nucleotide sequences flanking the introns are, however, very similar.

As a first step towards vector constructions, the promotor from one of the protein Z genomic clones will be fused to a GUS gene to test its activity in protoplasts of barley suspension cultures.

### Barley Peroxidases

Peroxidases are involved in many biological processes in plants. They are 1) tissue specific, developmentally regulated, or modulated by environmental stress factors; 2) they are involved in the biosynthesis of cell walls, in the regulation of auxin level, and in response to microbial attack; and they are 3) coded for by genes in at least three loci (*Prx1*, *Prx2* and *Prx4*) which are on barley chromosomes 5, 2 and 1, respectively. However, the exact position of the structural genes remains to be identified.

Three basic peroxidases (BP1, 2, and 3) from resting barley grains have been purified and antibodies produced. A cDNA clone encoding peroxidase BP1 has been isolated using antibodies. Hybridization to RNA from barley seeds collected every day from 10 to 25 days after flowering showed maximal expression of BP1 on day 15. A full-length cDNA clone was produced and the nucleotide sequence deduces 321 amino acids which shows less than 50 per cent sequence identity to those of dicot plant peroxidase, indicating that BP1 is a unique peroxidase. In collaboration with Novo Nordisk Ltd., recombinant strains of yeast are constructed to study the production of recombinant heme containing proteins.

Two cDNAs encoding peroxidases in barley leaves have been produced. An analysis of RNA from seedling leaves shows increased transcript levels for this cDNA four hours after inoculation with powdery mildew. The function of this peroxidase is studied in collaboration with the Plant Pathology Section, the Royal Vet.- and Agricul-

tural University, Copenhagen.

Cloning of the genes coding for the barley peroxidases aims at a characterization of sequences that specifies their tissue-specific expression, and of regions sensitive to stress factors. Well-characterized genes will be used to construct vectors for genetic transformation of barley. Genomic DNA libraries from barley have been screened for peroxidase genes using seed- and leaf-specific peroxidase cDNAs. Six genomic clones have been purified and the nucleotide sequence of one of them is analysed. The deduced amino acid sequence is corroborated by peptide sequences of peroxidase BP2. The BP2 peroxidase is 85 per cent similar to the amino acid sequence of peroxidase BP1.

This study is carried out in collaboration with J. Hejgaard, Technical University of Denmark, on protein purification, and K.G. Welinder, University of Copenhagen, on amino acid sequence analysis and biochemical analysis of the barley seed peroxidases.

### Regeneration of Plants from Barley Cells

The regeneration of normal plants from single cells is a prerequisite for producing transgenic crop plants, i.e. plants into which «foreign» genetic material has been transferred experimentally. Regeneration is a routine procedure in many dicot plant species, but it is difficult in monocots including the cereal species. The regeneration frequency of plants from one type of single barley cells viz isolated microspores has been improved by using a microblender for isolating spores and growing the cells on a solidified medium. It is now possible to regenerate plants from spring and winter varieties other than the model variety «Igri», where up to 600 green plants per spike can be produced. One hundred sixty-six anther-derived doubled haploid «Igri»-lines ( $R_2$ -generation) and chromosome-doubled haploid «Igri»-lines from microspore culture ( $R_1$ -generation) have been grown and evaluated in the field. Twenty-nine of the lines were morphologically diverging; 24 of them were autotetraploids. The 1000 kernel weight and grain yield of the non-morphologically deviating lines did not diverge significantly from that of the seed derived control lines.

### Embryogenic Barley Cell Suspension Cultures

To understand and improve the regeneration process, we have aimed at identifying extracellu-

lar proteins that are accumulated in the cell culture medium during the process where cells convert into embryos. These may subsequently develop into plants. Extracts were made from embryogenic and non-embryogenic cell-suspension media. The cell suspensions were initiated from embryogenic calli of immature embryos or microspore-derived embryoids. The analysis of the extracellular proteins by electrophoresis revealed 50 bands varying in size from 10 to 90 kD. Three of these bands have been identified as chitinase,  $\beta$ -1,3-glucanase and peroxidase. Proteins emerging only during embryogenesis from barley suspension cells have also been found.

With a monoclonal antibody two proteins with molecular weight 17 kD and 29 kD which occur only during embryogenesis have been isolated and characterized. The antibody can detect embryogenic development in cell cultures even before any differentiation is seen. In cooperation with Dianna Bowles, Leeds University, we found that the embryospecific antibody generated by her group recognized a 17 kD polypeptide different from that isolated by us. The embryospecific proteins are under chromatographic purification for sequence analysis.

Extracts of culture media from embryogenic cell cultures were used to »condition« a new culture medium for barley protoplasts. The »conditioned« medium optimized protoplast cultures, and the frequency of protoplast-colonies were enhanced up to 400 times.

The most recent development is the procurement of a microparticle accelerator. Isolated microspore cultures and protoplasts are used to study transient expression of introduced genes delivered to the cells either by the particle accelerator or by PEG (Polyethyleneglycols).

### 3.2 Resistance to Plant Diseases

Control of plant diseases by genetically controlled resistance is a cheap and environmentally safe protection measure for crop plants. It has become increasingly important in modern farming where diseased crop residues may not be removed completely from the soil surface and also in ecological farming where pesticides are not used and where economical and environmental concerns reduce the application of pesticides. Most of the work on plant diseases is concentrated on the resistance of barley to powdery mildew, because barley is an important crop plant, powdery mildew (Fig. 3.2.1) is an important bar-



Fig. 3.2.1. Colonies of the powdery mildew fungus on leaves of barley seedlings.

ley pathogen, and this host-pathogen system is easily amenable to research. In addition, other diseases of barley or other crop plants are studied. The main purposes of this research are to provide knowledge on the genetics and function of host-pathogen systems, to develop methods and techniques applicable to plant breeding, and to provide plant materials with diverse and, if possible, durable resistance.

#### Resistance to Barley Powdery Mildew

The work during the past year has dealt mainly with the identification of powdery mildew resistance genes in new commercial varieties, in breeding lines with less-well characterized resistance genes and in primitive lines of cultivated barley that may possess new resistance genes.

One hundred and twenty-seven spring barley varieties grown in Denmark since 1979 were analysed for the presence of resistance genes using 30 powdery mildew isolates and the pedigrees of the varieties. Eleven different, named genes and 12 tentatively named genes/resistances plus six unknown resistances were detected. Many varieties contain several of the earlier used genes in addition to new powdery mildew resistances/genes. The following new or relatively new resistance genes that are effective to the present powdery mildew population were detected: the »Mlo« resistance conferred by the recessive *mlo* gene, and »Ricardo« and »Turkish« resistances having gene *Mla3* in common and »Turkish« with *Ml(Tu2)* in addition. Three barley varieties have a new resistance, designated *Ml(IM9)*. The variety »Jarek« has two new so far unidentified resistances.

An analysis of the distribution of different

## Area in per cent

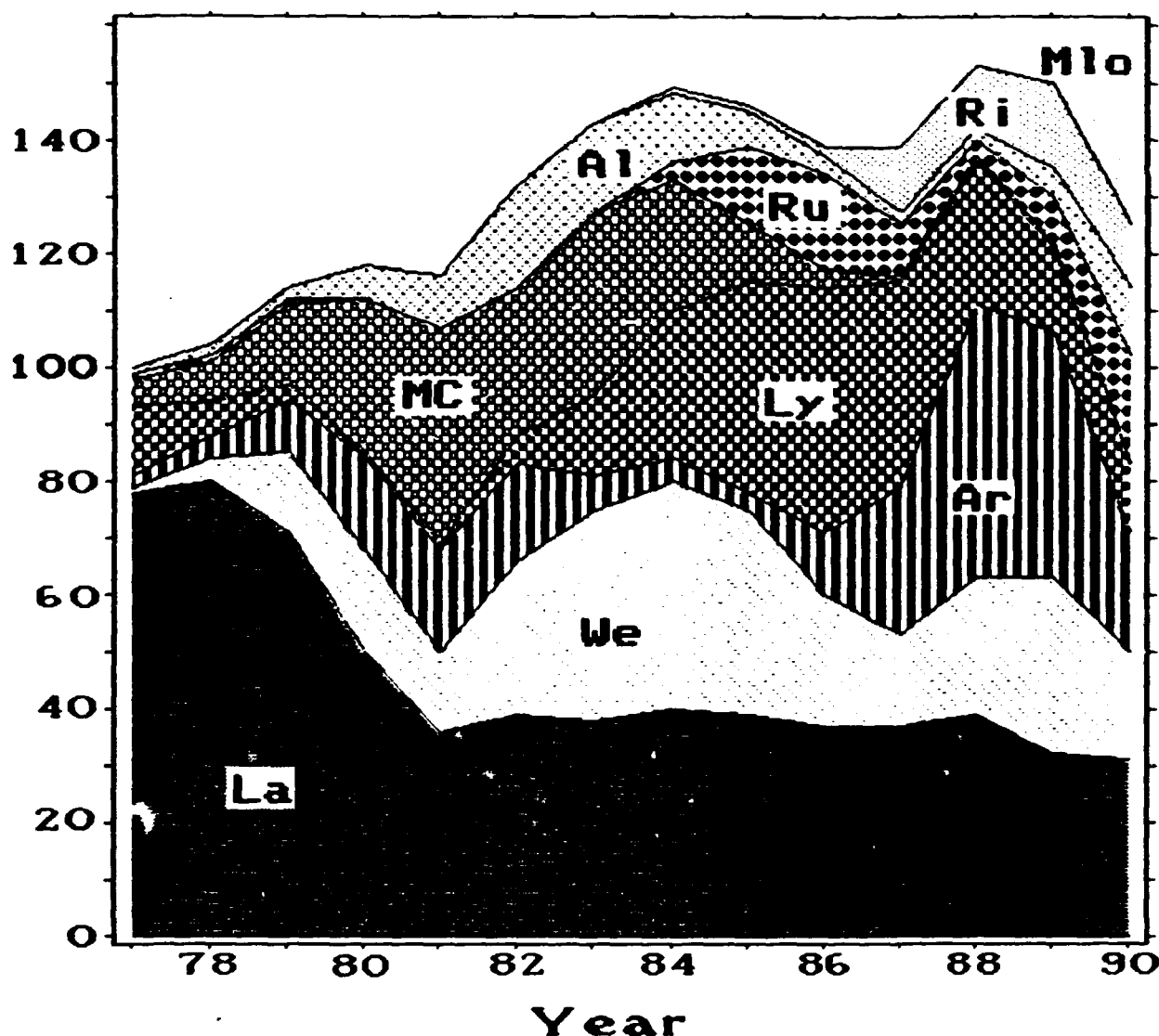


Fig. 3.2.2. Distribution on the Danish spring barley area of varieties grouped according to their powdery mildew resistance (La = *Laevigatum*, Ly = *Lyallpur*, MC = *Monte Cristo*, Ar = *Arabische*, Al = *Algerian*, Ru = *Rupee*, Ri = *Ricardo*, We = *Weihenstephan*, Mlo = *Mlo*).

powdery mildew resistances in Denmark (Fig. 3.2.2) reveals that the former extensive use of one or a few resistances (before 1980) has been replaced by a more diverse and extensive use of several resistances. A corresponding analysis of the frequencies of the virulences matching the above resistances in the Danish powdery mildew population revealed a close correlation between the frequency of resistance in the plant population and the frequency of the matching virulence in the pathogen population, though with a delay of one to five years for the virulences. It is noteworthy that the frequency of several virulences

declined following a drop in the frequency of the resistances, suggesting that formerly «defeated» resistance genes may become useful again.

Over the past seven years about 4,000 primitive lines or populations of cultivated barley have been screened for powdery mildew resistance in the field (Fig. 3.2.3). At present 19 lines appear to possess new, effective resistances; several lines have two or more resistance genes. By comparing these lines with those having known or formerly described powdery mildew resistance genes, we find that most of the 19 lines possess previously unknown resistance genes. The barley lines



Fig. 3.2.3. Severe attack of powdery mildew on barley lines from western Asia.

HORI1657 and HORI402 were found to possess two unique resistance genes, *Mla22* and *Mla23*, respectively, in locus *Mla* on barley chromosome 5. Further, the widely used (cf. Fig. 3.2.2) «*Lae-vigatum*» resistance gene *MI(La)* is linked to a gene for resistance to leaf stripe, but has not shown linkage to any of 49 genetic markers with known chromosomal locations.

#### Biocontrol of Powdery Mildew

The three-year SJVF-supported project concerned with the biocontrol of powdery mildew showed that the mirror yeast fungi (*Tilletiopsis* spp. - mainly *T. albescens*) are facultative hyperparasites on powdery mildew fungi. This means that, e.g. *T. albescens* grows on the leaves where it attacks and causes the collapse of the parasitic powdery mildew fungi when by chance it reaches the hyphae and conidia. The most effective *Tilletiopsis* species exude large amounts of constitutionally produced  $\beta$ -1,3-glucanase which may contribute to the collapse, as  $\beta$ -1,3-glucan is a cell wall constituent of the powdery mildew fungi. The relative humidity around the plants plays a decisive role for the use of *T. albescens* for biocontrol. More than 70 per cent relative humidity is necessary for a high and stable population of *T. albescens* on the leaves and for effective control of powdery mildew. For this reason, biocontrol with *T. albescens* in the field is unrealistic, whereas biocontrol of cucumber powdery mildew in greenhouses appears feasible (Knudsen and Skou 1990).

#### Resistance Mechanisms

Barley and other plants respond to pathogens or physical stress by forming structures called papillae between the plant cell wall and plasma membrane. Barley plants with *Mlo* powdery mildew resistance develop very few if any mildew colonies when inoculated with powdery mildew. The *Mlo*-resistant plants produce papillae that are twice as large as those in the mother varieties. Furthermore, papillae are detectable earlier and develop at a higher rate. The main component of the papillae is the polysaccharide callose, a 1,3- $\beta$ -linked glucan. Callose is synthesized by the plasma membrane-associated enzyme 1,3- $\beta$ -D-glucan synthase (1,3-GS) with UDP-glucose as the substrate. A time-course study was conducted lasting 30 hours starting from inoculation with powdery mildew of a near-isogenic pair of barley lines with and without gene *mlo5*. Non-inoculated plants served as control. Epidermis strips from the barley leaves were collected. Some strips were stained with the callose-specific stain lacmoid, and the diameter of the papillae was then measured; from other epidermal strips the microsomal fraction was isolated and the 1,3-GS activity was measured. The results showed no difference in specific 1,3-GS activity between the inoculated *Mlo*-resistant and the susceptible lines. Nor was there any difference in the specific 1,3-GS activity between the inoculated and non-inoculated plants. The lacmoid staining confirmed that the callose is synthesized earlier and with a higher rate in the resistant line than in the susceptible one. The callose in the papillae thus appears to be synthesized by pre-existing 1,3-GS. A final conclusion may be drawn when the enzyme is purified and the amounts of the 1,3-GS enzyme present in both the resistant and susceptible lines have been measured.

1,3-GS was solubilized from barley plasma membranes with the detergent digitonin, separated on a 5-30 per cent sucrose gradient, and the enzyme was purified 5-15 times. Further purification was achieved by making product entrapment. Sucrose gradient fractions with high specific activity were pooled, UDP-glucose was added and the enzyme and its product were precipitated. Thereby the purification was about 100-150 times. SDS-PAGE of the product-entrapped enzyme showed enrichment of 3-6 polypeptides with molecular weights ranging from 18 to 170 kD. Apart from these, some weakly stained poly-

peptides could be detected. The 1,3-GS has not yet been purified to homogeneity in any plant species, but purification is in progress in four other laboratories.

In barley, expression of chitinase isoforms has been concluded to be strictly tissue-specific on the basis of purification experiments and immunoblotting after SDS-PAGE. In young healthy leaves only chitinase 1 is detected. This isoform is localized intracellularly and is not induced upon powdery mildew attack. In contrast, a 27 kD protein which reacts with the chitinase antibodies is strongly induced upon powdery mildew infection. It is currently being tested whether this protein which preferentially is present in the intercellular space of the leaves is a chitinase. In barley grain the chitinases K, T and C are found, but not chitinase 1 nor the 27 kD protein. In a barley embryogenic suspension culture chitinase K, T, C and 1 were expressed. Stress treatment of the suspension cells induced the chitinase K and/or T only weakly. In conclusion, expression of chitinases in barley appears to be controlled developmentally and/or to be stress-induced in a tissue-specific manner. The enzymes may serve as preformed or induced antifungal defence mechanisms, however, alternative endogenous functions cannot be excluded. This project was performed in association with Susanne Jacobsen, Technical University of Denmark, and Jørn D. Mikkelsen, DANISCO.

In another study rapeseed leaves infected with *Phoma lingam*, the dry rot and canker fungus, showed a 14-fold increase in chitinase activity over an 8-day period. Crude extract of *P. lingam* as well as the liquid growth medium from fungal cultures were assayed for chitinase activity, but enzyme activity was not found in the medium, and only minor activity in the extract from the fungus itself. Therefore, the increased chitinase activity in infected rapeseed leaves must be due to plant synthesis of the enzymes. Four isozymes, three minor and one major fraction, have been purified from *P. lingam* infected rapeseed leaves by affinity and ion exchange chromatography. The major fraction, a basic chitinase with a molecular weight of 30 kD was strongly induced after infection. A partial amino acid sequence of this polypeptide is being determined. A cDNA library from *P. lingam* infected leaves has been constructed and screened with antibodies specific against the 30 kD polypeptide. Five different positive clones have been isolated and partly se-

quenced. No homology has been found to known chitinase genes.

### Barley Leaf Stripe

One hundred and ten modern spring barley varieties and breeding lines were tested for resistance to barley leaf stripe (*Drechslera graminea*) in order to update former screenings of about 1,000 mainly European barley varieties and lines. The results confirm that a gene for high resistance to leaf stripe is frequently present in commercial varieties and advanced breeding lines, but is usually unnoticed. In fact, 32 per cent of the barley area in Denmark in 1990 was grown with resistant varieties. We suggest that seed treatment of highly leaf stripe-resistant varieties with fungicides is superfluous.

### Pathogen Genetics

In the past few years we have intensified our studies on the genetics and molecular biology of the powdery mildew fungus. The present report focuses on five topics: linkage analyses, chromosome number, gene expression, plasmids and Mlo aggressiveness.

Linkage analyses of 80 progeny isolates from a cross between barley powdery mildew fungal isolates have been carried out using 31 RFLP markers and five virulence loci. Five linkage groups have been established, two of which show linkage between RFLP markers and virulence loci. Many of the RFLP markers represent multicopy sequences in the fungal genome and are present on different chromosomes. They cannot be used as starting points for chromosome-walking in an attempt to isolate the specific avirulence genes. Single copy sequences have not detected any polymorphisms by the conventional method. We have adopted a method from a German laboratory based on long polyacrylamide gels and restriction enzymes which recognize four base pairs. This facilitates the detection of polymorphism using single copy sequences. Linkage analyses of RFLPs representing single copy sequences are being carried out in an attempt to find a marker very closely linked to an avirulence gene.

The chromosome number of the barley powdery mildew fungus has been examined using cytological techniques and pulse field gel electrophoresis. An improved staining method for the

fungal chromosomes has been developed and the haploid chromosomal complement has been estimated to be at least seven. Attempts at electrophoretic separation of chromosomes resulted in the detection of five high molecular weight DNA bands in a hybridization experiment using RFLP markers; the bands were confirmed to represent chromosomes. Chromosomal DNA isolated from pulse field gels has been inserted into YAC (Yeast Artificial Chromosome) vectors and used for transforming yeast cells. Only few clones have been obtained and optimization of the system is required.

A cosmid library of powdery mildew fungal DNA has been screened for sequences that were expressed by using labelled mRNA isolated from mildew-infected barley plants. All fungal sequences that were found to be expressed in this way also showed homology to pure barley mRNA. These sequences probably represent conserved genes encoding metabolic proteins common to both organisms. This method of selection is not sensitive enough to obtain fungal sequences that are expressed during the early infection process. Attempts are being made to obtain pure fungal RNA. All the expressed sequences have been found to be bordered by DNA segments repeated many times in the fungal genome, but not present in the barley genome. Coding and non-coding repetitive sequences have been separated by subcloning to examine the diversity of repeated sequences bordering the coding regions. Selected repeated sequences will be sequenced to determine whether they show homology to transposable genetic elements identified in many fungal genomes.

A mitochondrial plasmid present in many powdery mildew isolates has been cloned and sequence analysis have shown the presence of terminal inverted repeat elements characteristic of fungal linear plasmids. The plasmid contains sequences that are expressed, and sequence analysis are presently being carried out to identify the expressed genes. Mitochondrial DNA has been cloned and a large number of fungal isolates analysed for polymorphism. Only one isolate differed indicating a very conserved mitochondrial genome. The inheritance of mitochondria and plasmids was studied in a cross between two isolates differing both in mitochondrial genotype and with respect to the amount of plasmid present. The results indicated that in the same fungal isolate, mitochondria with and without



Fig. 3.2.4. Seedling leaves of *Mlo* resistant (left) and non-resistant (right) barley inoculated with an *Mlo*-aggressive powdery mildew isolate.

plasmids were present. It also appeared that plasmids were present in mitochondria of different genotypes.

The *mlo* powdery mildew resistance gene has become widely used in European barley breeding and production. A 3-year study supported by «Internordic Plant Breeding» on the *Mlo* aggressiveness of the powdery mildew fungus was completed. Methods were developed for a routine surveying of natural powdery mildew populations for the presence of elevated levels of *Mlo* aggressiveness (Fig. 3.2.4). No signs of increasing levels of *Mlo* aggressiveness were found, either in Danish or continental European powdery mildew populations.

### Fungal Pathogens on Other Organisms

The expertise at Risø on fungal pathogens of crop plants has since long time been extended to fungal pathogens on other organisms, particularly on bees that are important for pollination and subsequent seed setting in some crop plants. Several new bee pathogenic fungal species now have been identified. In the past, xerophilic *Chrysosporium* species often were isolated along with *Ascosphaera* species at the sites of solitary bees used for pollination. Studies of the physiology reflect the special habitat of these fungi. They grow very sparsely on common fungal media, but their growth rate and sporulation increase with the glucose content up to about 40-50 per cent and they even grow between the crystals in media so concentrated that glucose crystals are formed.



## 4 Nutrient Efficiency in Plant Production

Agricultural plant production interacts with the quality of the water-, soil-, and air environment. The decomposition of crop residues and farmyard manure constitutes a risk of leaching mineral elements, especially N to the groundwater, lakes and seas. Furthermore, the production and distribution of fertilizers require large amounts of energy resources. A diverse population of soil microorganisms is involved in the cycling of mineral nutrients. Improved knowledge of the microbial decomposition of crop residues, farmyard manure, and other organic matter is important not only for maximizing the quantity and quality of nutrients given to the plants, but also in order to manage the potential loss of nutrients. Certain soil microorganisms establish symbiotic relations with the crop plant; *Rhizobium* bacteria fix atmospheric nitrogen in symbioses with legumes, while VA-mycorrhizal fungi establish symbioses with a range of crop plants supporting the plant uptake of mineral elements from the soil. An extensive research effort is required to elucidate the many complex processes that regulate the circulation of mineral elements in the soil-plant-air environment in agriculture. The aim is to devise crop production strategies that consider crop productivity, the quality of plant products, and environment.

### 4.1 Nitrogen in Soil and Plants

Our aim is to elucidate the intricate biological, physical and chemical processes involved in the mineral element cycles in the soil-plant-air system. At present, the main emphasis is on processes involved in the cycling of nitrogen (N) in crop residues, catch crops, farmyard manure and mineral fertilizers.

#### Fate of N from Crop Residues and Manure

Nitrogen mineralized from crop residues and farmyard manure contributes to the nitrogen nutrition of succeeding crops, but may also be lost by leaching and denitrification. The release and fate of N from  $^{15}\text{N}$  labelled catch crop material, incorporated in December, were studied in microplots in the field. In April the following year, 45 and 85 per cent of the  $^{15}\text{N}$  from white mustard

and perennial ryegrass catch crops, respectively, remained in organic residues in the top soil. Measurement of leaching below 45 cm soil showed that less than 10 per cent of the ryegrass catch crop N was lost by leaching during the mild winter 1987/88. After one year of decomposition 60 and 47 per cent of the mustard and ryegrass N, respectively, were released. The faster release of N from mustard than from ryegrass material was probably due to the higher N content of mustard than of ryegrass. Spring barley cropped the following three years after the incorporation of ryegrass N recovered 19, 4 and 2 per cent, respectively, of the labelled N. A 2-year ryegrass sward recovered 26 per cent of the labelled ryegrass N. A mass balance of labelled ryegrass N after one year of decomposition in unplanted soil indicated gaseous N losses of 13 per cent.

The mineralization and uptake in succeeding crops of  $^{15}\text{N}$  labelled N from pea and barley straw with 2.8 and 0.9 per cent N, respectively, were studied in an outdoor pot experiment. Incorporating pea and barley straw reduced the N accumulation in an autumn grown catch crop. The catch crop recovered 11 and 3 per cent of the pea and barley straw N, respectively. A succeeding spring barley crop recovered 13 and 10 per cent of the pea and barley straw N, respectively. If pea straw was incorporated in the spring, 18 per cent of the N was recovered by spring barley. Straw incorporation did not influence total N accumulation in spring barley. The results confirm results from the field, showing that 15 to 20 per cent of the pea straw N is recovered in a succeeding crop.

Studies on the mineralization of N from the organic fraction of farmyard manure have been initiated. A method for labelling the manure with  $^{15}\text{N}$  is being evaluated. A ruminant is fed with labelled ryegrass for nine days. The uniformity of labelling is evaluated before the labelled manure is used in microplot experiments.

#### Denitrification

The microbial reduction of nitrate and nitrite to gaseous nitrogen (denitrification) is the major biological process in which industrially and biologically fixed N is returned from the soil into the atmosphere. Growing plants influence denitrifi-

cation and may stimulate or depress the process depending on the environmental conditions. The influence of pea (*Pisum sativum* L.) and spring barley (*Hordeum vulgare* L.) on denitrification was investigated in closed pots flushed with air of known composition. Denitrification was estimated by means of the acetylene inhibition technique and the effect of plant age, soil nitrate and soil aeration status was investigated. The highest denitrification rates were measured in soil planted with pea, followed by soil with barley plants, and lowest in unplanted soil. Denitrification increased with increasing plant age. The addition of nitrate had only a small effect on denitrification in soil with 3 week-old plants and in unplanted soil, whereas denitrification increased 10 fold after nitrate addition in soil with 5 week-old plants. The results emphasize that organic carbon (C) is a major controlling factor in soil denitrification. It is assumed that more available organic C in the root deposits of pea relative to barley is responsible for the higher denitrification in soil planted with pea. Higher oxygen (O) consumption due to respiration by heterotrophic organisms in the rhizosphere of pea makes denitrification in soil planted with pea relatively less sensitive to the O concentration compared with soil planted with barley and unplanted soil.

Quantification of denitrification in the field is very difficult due to the high spatial and temporal variability. In a mass balance study  $^{15}\text{N}$ -labelled nitrate added to soil grown pea or barley were compared with respect to  $^{15}\text{N}$  unaccounted. During the growing season of 1989, which was drier than average, only negligible N losses due to denitrification were measured by means of the acetylene inhibition technique. A substantial amount of fertilizer N was unaccounted for by the  $^{15}\text{N}$  mass balance, especially in the pea plots. The loss took place during the period of grain filling where no leaching occurred and was accompanied by a decrease in the  $^{15}\text{N}$  content of the plants; volatilization of ammonia from the aerial parts of the plants is a possible explanation of the observed loss.

## 4.2 Root-Microbe Symbioses

Symbiotic associations between plants and soil-microorganisms are of major importance to plant nutrition. Our research in this field includes the physiology and ecology of the legume *Rhizobium* symbiosis and VA-mycorrhiza, a symbiosis between plants and fungi.

### Symbiotic N Fixation

The ability of legumes to establish a symbiotic relationship with *Rhizobium* bacteria able to fix atmospheric nitrogen into ammonia accessible to plants has been essential in sustaining the fertility of agricultural land for centuries. Symbiotic N fixation is fueled by products from the photosynthetic activity of the plant. This input of nitrogen into the crops is derived from solar energy, rather than from the fossil energy used to produce nitrogen fertilizer.

In the mature legume nodule, the *Rhizobium* bacteroids are enclosed by a plant-derived peribacteroid membrane (PBM). Information on the transport of compounds across the PBM is valuable because it may explain how this mutualistic relationship differs from a parasitic one. The PBM may also be essential for regulating of  $\text{N}_2$  fixation by controlling the exchange of compounds between the symbionts. In collaboration with colleagues from Murdoch University, Western Australia, we have made preparations of bacteroids enclosed by an intact PBM from pea and soybean nodules (Fig. 4.2.1) in an anaerobic environment. The resulting metabolically active peribacteroid units (PBU) have been used to investigate the exchange of compounds between the symbionts. The PBUs were incubated in a reaction mixture containing a  $^{14}\text{C}$  labelled compound as the sole C substrate or supplemented by a second unlabelled C substrate. The fate of the  $^{14}\text{C}$  was determined within the PBUs as well as in the reaction mixture. By this technique we have demonstrated the existence of an exchange of C between the organic acid and the amino acid pool associated with the interface of the symbionts. Our results indicate a more extensive metabolite exchange between the symbionts than originally assumed, and suggest that transaminase activity is associated with the PBUs.

Investigations on the ecology of rhizobia showed that a *Rhizobium* population on pea had a highly correlated variation in genetic markers on the symbiotic plasmid and on the bacterial chromosome (Engvild *et al.* 1990). This indicates that there is a limited exchange of plasmids between bacterial strains under natural conditions. It is consistent with observations that most bacterial populations have a clonal population structure, shaped primarily by selection, mutation and genetic drift. The recently observed transmissible antibiotic resistance in natural bacterial po-



Fig. 4.2.1. Electron micrographs of PBUs from A: Pea and B: Soybean nodules. Large arrows point to PBM, small arrows point to the bacteroid membrane. Bars represent 2  $\mu\text{m}$ .

pulations is caused by a strong selection pressure and should not be generalized.

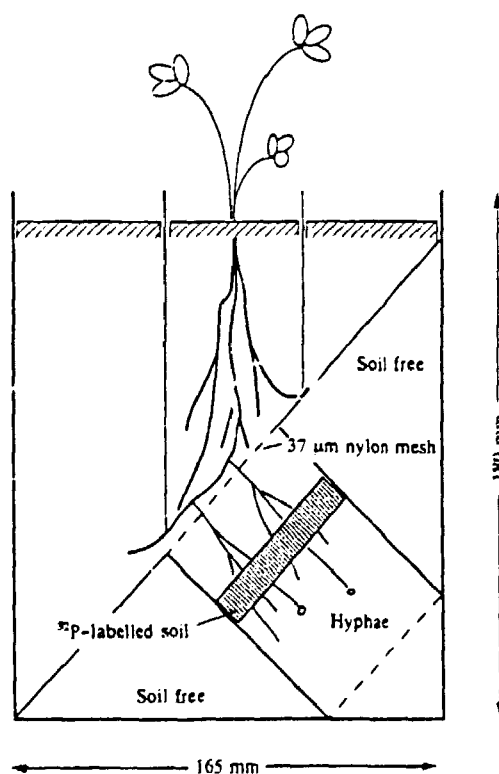
#### Mycorrhizas and Plant Nutrition

The formation of vesicular-arbuscular (VA) mycorrhiza between plant roots and certain soil fungi has a beneficial impact on the nutrient uptake by the plants. The nutrients in the soil are absorbed and transported to the roots by the external fungal hyphae. The phosphorus and nitrogen nutrition of VA-mycorrhizas are studied by means of a two-compartment system, which makes it possible to distinguish hyphal nutrient uptake from direct uptake by the roots. A hyphal compartment is separated from the root compartment by a fine mesh, which does not restrict growth, but allows free passage of the hyphae (Fig. 4.2.2). Isotopes placed in the hyphal compartment are used as tracers.

The relationship between phosphorus (P) uptake by mycorrhizal *Trifolium subterraneum* and the spatial distribution of the external hyphae of three mycorrhizal fungi, *Scutellospora calospora* (SC), *Acaulospora laevis* (AL), and *Glomus* sp. (GL), has been studied in collaboration with researchers in Department of Soil Science and Plant Nutrition, the University of Western Australia. Soil samples labelled with  $^{32}\text{P}$  were placed at different distances from the roots and the

radioactivity in hyphae and plant components was measured after five weeks. The spread of hyphae differed considerably between the three

Fig. 4.2.2. Two-compartment system used to study  $^{32}\text{P}$  uptake by external hyphae of VA-mycorrhizas.



fungi used. The hyphal density of SC declined exponentially with distance from the roots, whereas AL maintained a constant hyphal density over the full distance of spread. Hyphae of GL were formed at a constant plateau close to the roots and then declined exponentially with distance. The rate of spread of AL was approximately 3 mm day<sup>-1</sup>. The P uptake by AL and GL was closely related to the distribution of the external hyphae. Significant amounts of radioactivity were detected in the host plant when the <sup>32</sup>P was placed 70 mm from the roots. Plants colonized by SC contained very little radioactivity, but the specific content of <sup>32</sup>P in SC hyphae was three times as high as in AL and GL hyphae. This indicates that either hyphal P translocation or P transfer across the host-fungus interface was inefficient in the association with SC. These results show that the capacity of a VA-mycorrhizal fungus for supplying P to the plant is influenced both by the rate of hyphal spread, and by the capacity of the hyphae to translocate or transfer P to the host (Jakobsen *et al.* 1990).

In contrast to the well-established importance of VA-mycorrhizas in phosphorus nutrition the hyphal uptake and transport of soil nitrogen have been investigated only slightly. Cucumber was grown with or without the VA-mycorrhizal fungus *Glomus fasciculatum* in a two-compartment system. After the establishment of a hyphal network, <sup>15</sup>N labelled ammonium was added to the soil in the hyphal compartment 5 cm from the roots, and the presence of <sup>15</sup>N in the hyphae and plant parts was subsequently measured. Previous problems with lateral movements of <sup>15</sup>N to non-mycorrhizal plants were avoided by using N-serve, a nitrification inhibitor. In mycorrhizal plants, 6 % of the added <sup>15</sup>N was translocated to the plant via the hyphae. Purified external mycelium had an enrichment of 1.2 atm. per cent <sup>15</sup>N showing that a considerable part of the labelled N source was localized in the external mycelium. The atm. per cent <sup>15</sup>N in the hyphae was only 0.3 when nitrification was not inhibited indicating a preference for ammonium over nitrate. The N uptake by hyphae could also be detected as a depletion of inorganic N in the root-free compartment. In the presence and absence of mycorrhiza, soil 5 cm from the root system contained 2 and 35 ppm inorganic N, respectively. Experiments with barley showed an N uptake pattern close to that of cucumber. This demonstration of a significant absorption and transloca-

tion of soil nitrogen by VA mycorrhizal hyphae and the subsequent transfer of nitrogen to the host plant was obtained with a suboptimal N supply to the plants.

Our work on mycorrhizas is carried out as part of a joint research programme on microbial processes in the root zone. The programme was initiated by the Danish Agricultural and Veterinary Research Council and involves seven different research laboratories in Denmark.

### 4.3 Influence of Plant Variety on the Root Uptake of Radiocaesium

Elements other than plant nutrients, such as heavy metals and fall-out products, are taken up by crop plants. The study of these processes is therefore necessary in the elucidation of the plant-soil environment. Studies of genetic differences in plant uptake of radiocaesium, in collaboration with the Section of Plant Biology at Risø, were concluded with a pot experiment.

Four varieties of spring barley and three varieties of rye-grass have been tested for their sensitivity to indirect caesium contamination, i.e. an absorption through the root system of caesium that has entered the soil. Ten different combinations of soil type, caesium isotope and age of caesium contamination were studied in this experiment during the years 1988, 1989 and 1990.

The results for barley grain are shown in the Table 4.3.1. Analyses of variance of these results showed a significant difference between the four varieties in each type of soil and for each isotope, confirming a high root uptake of radiocaesium in »Sila« and a significantly lower root uptake in »Apex«. The pattern between the different varieties was identical in each of the three years.

Similarly for the grass varieties, one variety, the Italian rye grass, was identified in all cases as having the relatively highest uptake of radiocaesium.

The total uptake decreased over time due to fixation of caesium in the soil.

In summary, it might be possible, to reduce the radiological consequences after a nuclear accident by using plant species and varieties with low sensitivities to a given contamination.

The conclusions given here might have a wider perspective concerning indirect contamination with heavy metals. This should be studied further.

**Table 4.3.1. Root uptake of radiocaesium in barley grain expressed relative to the concentration ratio for the variety Apex in each group (year-soil-isotope category). Each value is the mean of four replicates  $\pm$  1SE**

Soil and isotope	Variety	1988	1989	1990
Organic soil experimentally added Ca-134	Apex	100 $\pm$ 7%	100 $\pm$ 8%	100 $\pm$ 15%
	Golf	101 $\pm$ 10%	105 $\pm$ 7%	82 $\pm$ 12%
	Anker	128 $\pm$ 7%	150 $\pm$ 13%	120 $\pm$ 11%
	Sila	136 $\pm$ 7%	121 $\pm$ 19%	167 $\pm$ 20%
Organic soil Chernobyl Ca-137	Apex	100 $\pm$ 7%	100 $\pm$ 13%	100 $\pm$ 20%
	Golf	102 $\pm$ 10%	84 $\pm$ 8%	82 $\pm$ 11%
	Anker	132 $\pm$ 6%	115 $\pm$ 13%	114 $\pm$ 5%
	Sila	154 $\pm$ 9%	112 $\pm$ 16%	159 $\pm$ 18%
Sandy-loam experimentally added Ca-137	Apex	100 $\pm$ 9%	100 $\pm$ 10%	100 $\pm$ 7%
	Golf	109 $\pm$ 5%	140 $\pm$ 5%	112 $\pm$ 3%
	Anker	116 $\pm$ 7%	152 $\pm$ 4%	124 $\pm$ 4%
	Sila	134 $\pm$ 4%	204 $\pm$ 7%	139 $\pm$ 5%

## 5 Chemistry of the Geosphere

### 5.1 Geochemical Modelling

An interest of long standing in chemical mechanisms and in processes influencing the spreading of contaminating materials, has led to the development of PC programs for calculating equilibria in complicated geochemical systems.

Two contracts, »Geochemical Modelling« and »Geochemical Databases«, both within the frame of the EC nuclear research programme, were concluded during 1990 with the completion of the following reports:

**PMATCH: A Program to Manage Thermochemical Data.** This program is used for adjusting the limited data of varied quality, which is available from the literature, to the same frame of reference, and to secure that the data will be internally consistent with other data in the database.

**LITTLE JOE, An Expert System to Support Geochemical Modelling.** This program can be used for the evaluation of analytical data for ground waters from limestone formations.

**JENSEN, A Program for the Computation of Chemical Equilibria in Aqueous Systems.** This program has now been developed to the stage where practical problems can be solved e.g. the speciation of components in moderately saline solutions. The program also calculate which minerals and how much of each will precipitate under given circumstances. The program is under continuous development as to databases and new processes.

Several minor programs, **SOLIDSOL**, for the computation of equilibria involving solid solutions among minerals, and **SOLSAT**, a rapid method for computation of equilibria where pure solid phases are involved, have been developed as well as a program for calculation and displaying **STABILITY DIAGRAMS** for redox-active elements like sulphur, iron, manganese and others.

Two new EC contracts for the period 1991-1993 have been negotiated and accepted, »The Role of Colloids in the Migration of Radioelements« and »Uncertainties in the Modelling of Migration«.

## 5.2 Applied Geochemistry

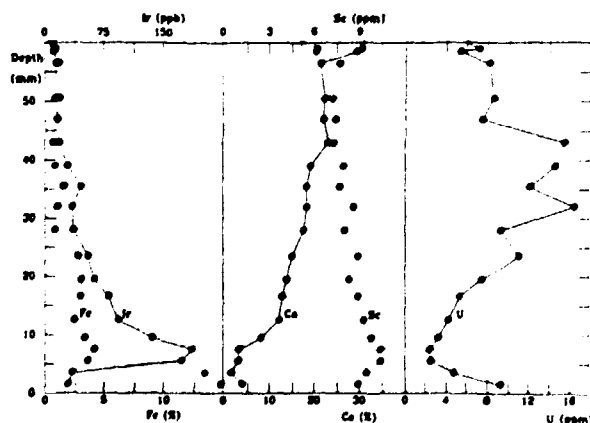
### Methods

The main area of interest is the evaluation of geochemical data obtained by various analytical techniques at Risø. Most of the work is carried out in close cooperation with other, mainly geological institutions. The group commercial analyses (X-ray fluorescence, gamma spectrometry) are carried out for customers in Denmark and abroad, and with the company Tracechem, a joint contribution describing a new instrumental neutron activation technique for the analysis of large samples was presented at the conference EUROANALYSI VII in Vienna. The method proposed is based on the direct irradiation of several 7-ml polyethylene containers followed by counting in an array positioned around a Ge(Li) detector. The analytical method is especially suited for the analysis of large (up to about 60 g) heavy-metal polluted samples.

### Terrestrial Geochemistry

A systematic study of the uranium geochemistry close to the Cretaceous-Tertiary boundary was terminated and published in 1990. The boundary Fish Clay characterized by high contents of the metal iridium and marking the extinction of several planktonic foraminifera, calcareous nannoplankton and dinosaurs is found at several places around the Danish territory. The investigation which is part of an ongoing joint effort (funded

*Fig. 5.2.1. Distribution of U, Ca, Fe, Sc and Ir with depth in the Fish Clay from Stevns Klint (locality Harvig). Note that U does not correlate with any of the other plotted elements.*



by SNF) with the Geological Institute of the University of Copenhagen (Hans Jørgen Hansen) and DGU (Jens Morten Hansen) lead among others to the conclusion that due to the relatively low U contents close to the boundary uranium toxicity can be ruled out as a cause of the extinctions.

Together with the University College Galway, Ireland (Martin Feely), a study of the rare earth element geochemistry of Irish late-Caledonian granites was continued. These granites may be characterized according to their normalized rare earth element patterns. To some extent the late-stage overprint of hydrothermal processes is visualized in special REE patterns.

### Marine Geochemistry

Scientific cooperation with the Kiel University (prof. P. Stoffers) and the New Zealand Oceanographic Institute (G.P. Glasby) continued with main emphasis on the occurrences of heavy metals in marine ferromanganese deposits. Joint papers were written regarding the geochemistry of newly discovered manganese crusts and nodules from the Philippine Sea. Own input into this work was mainly in the form of investigations regarding the rare earth element (REE) geochemistry in the ferromanganese material. In 1990 practical work ended with an energy research project »Time-temperature variations in North Sea Central Trough sediments as revealed by fission track determinations of selected drill cores« (EFP-88). A final report will be prepared within the first part of 1991. The project, which is a joint effort with the Institute of Petrology, University of Copenhagen, and the Risø reservoir group was, in 1990, characterized by analytical work on cutting samples from 2 wells from the Danish Central Trough, 1 well in an adjacent area and 1 well from the deeper parts of the Danish Subbasin. The technique applied makes use of the thermal shortening of tracks generated in the mineral apatite during the spontaneous fission of  $^{238}\text{U}$ . In this respect, the track length is an indicator for the temperature a mineral has been opposed to within the sedimentary sequence. Knowledge of this temperature in turn is important for evaluating the hydrocarbon potential of a sedimentary basin. A numerical model based on fission track length frequency distributions was developed. In 1990, most of the results of the study were presented at international meetings in Copenhagen, Germany and Australia.

## 5.3 Geochemical Technology

### Fixation of Heavy Metals in Soil

It is well-known that clay minerals can bind soluble cationic metal ions by functioning as ion exchangers, and that many of these metal ions can become much more firmly bound to the clay by entering into the tetrahedral or octahedral layers of clay minerals. If an alkaline clayey suspension containing heavy metal ions is heated at 260°C under pressure such as fixation of the metal ions may be produced, and the metal cations can no longer be leached from the clay at pH-values  $\geq 3$ . However, the presence of organic compounds forming negatively charged metal complexes may impede the fixation.

Some cations e.g.  $\text{Cd}^{++}$  fit poorly into the clay mineral lattice and they are retained less firmly. Also, soil contains other materials with cation-exchange properties e.g. ferric hydroxide and hydrous manganese dioxide, and these may compete with clay minerals for the metal ions without establishing the acid-proof fixation.

Our current test method only simulates leaching at different pH, but plant roots are known to take up nutrients that are hard to get at. For examination of the interaction between treated soils and plant roots, pot experiments have been envisaged in collaboration with plant biologists.

An alternative fixation method consists in hydrothermal sulphidizing. This process is carried out by autoclaving soil under anoxic conditions in the presence of a small amount of sulphur. Associated with this study are experiments on the possible bacterial redissolving of sulphides under a different oxygen pressure.

This long term project is dependent of financial support, e.g. from the EC STEP programme.

### Wet Oxidation of Organic Material

Our continued collaboration with NKT Ltd. has resulted in a number of wet oxidation procedures for solving various specified problems. For a better understanding of the reaction mechanism, a series of laboratory tests has been carried out on wet oxidation of lower fatty acids with special attention to formic acid. Formic acid exhibits a parallel decomposition into  $\text{CO}_2$  and  $\text{H}_2$  which seems to explain the intriguing occurrence of hydrogen during wet oxidation of polymers such as polyethene.

Also the decomposition of PVC has been investigated. The reaction products appear to be non-toxic and biodegradable.

### Marine Environmental Technology

The Applied Geochemistry group in cooperation with Roskilde University (RUC, Institute of the Environment, Technology and the Society), Center for Advanced Technology (CAT) and Danish industries worked with projects related to the environmentally safe offshore recovery of sand and gravel in Danish sea territories. At present the establishment of a Marine Mineral Technology Center is envisaged. In 1990, a feasibility project regarding optimal and safe offshore exploration techniques for heavy minerals was partly funded by the Danish authorities (Industri- og Handelsstyrelsen). The project work included the construction of the prototype of a marine probe in cooperation with the Risø Electronics Department. Project proposals were prepared for forthcoming national and EEC research programs.

### Corrosion Tests of Ceramics Materials

In collaboration with a private firm Viking Chemicals a series of corrosion tests are being carried out on  $\text{ZrO}_2$ -based ceramics with reference to their application for implants such as artificial hip joints. The test conditions are simulating body fluids at the temperature of 37°C. Results are obtained within a reasonable time thanks to the high sensitivity of the available analytical method (ICP/MS).

### SANS-Measurements on Cementitious Materials

A contract within the frame of The Third EC R&D programme on »Management and Disposal of Radioactive Waste«, titled »Characterization of Low- and Medium-Level Waste Forms« was completed in 1990. All work was carried out in collaboration with the Risø Waste Management Section.

One of the topics in the contract was a study of cements using Small Angle Neutron Scattering, a useful technique for characterizing microstructures in the nanometer(nm)-size range.

Cements when hydrated and hardened contain rigid calcium-silicate (CSH) structures of gel-particles, average size about 10 nm with numerous,

interstitial sometimes liquid-filled pores varying in size between 1 nm and 100 nm.

A SANS at Harwell on the formation of the CSH-phase in various cementitious mixtures lead to the suggestion that CSH-gel formation in cement is taking place by way of single particle diffusion-limited aggregation of calcium-silicate-gel globules, and that a self-similar structure with a fractal dimension of  $\sim 2.4$  is the result. Our findings are in agreement with this suggestion.

Cements are quite susceptible in time to chemical and physical changes affecting the microstructure of the cement, and the aim of the Risø SANS-study is to carry out structural studies of cement paste undergoing degradation in various ways. The experimental methods are by now well established and tentative results indicating coarsening effects on the gel-structure have been obtained. The SANS studies on the degradation of the CSH-phase in various cementitious mixtures will be continued within the frame of the EC R&D programme for 1990-1994 under a new contract titled »The Performance of Cementitious Barriers in Repositories«.

#### Integral Experiments

A series of year-long experiments determining the rate of release of additives as well as other components from cement through layers of clay, chalk and sand have been carried out, and a PC-program DIFMIF2, written in C has been validated using the experimental results with kaolin as a barrier. DIFMIF2 calculates the one-dimensional diffusive migration of single substances through multi-barrier systems according to the diffusion equation,  $\delta C/\delta t = D_c(t) * \delta^2 C/\delta x^2 + F(C,t)$ .  $F(C,t)$  is a function responsible for time-dependent changes in the concentration. DIFMIF2 is at the moment being expanded to include e.g. precipitation reactions.

Integral experiments for studying the diffusive migration through barriers of inert materials will continue under our previously mentioned EC contract for 1990-1994.

## 5.4 General and Inorganic Chemical Analysis

Chemical analysis have been carried out mainly by atomic absorption (AA), ionchromatography (IC) and inductively-coupled plasma mass spec-

trometry (ICP/MS). The PlasmaQuad from VG Elemental Ltd. was until recently the only instrument of its kind in Denmark and evaluation of ICP/MS methods has had a high priority since its installation in 1987. The two methods ICP/MS and AA supplement each other well and have been used in a wide variety of projects being carried out at Risø e.g. to gain information of trace elements on soil-decontamination processes, in processes developed for extraction of elements from a variety of matrices, in ecological projects on rainwater and in studies on environmental samples from Greenland and from Chernobyl. Of special interest is the project »Metodik til måling af tilgængelighed af sporelementer i levnedsmidler og kost« supervised by research professor Brittmarie Sandström, KVL. The mass spectrometric detection method in ICP/MS opens up the possibility of using non-radioactive enriched isotopes in risk-free studies involving animals and human beings. In the first experiments selected pigs have been given fodder with addition of  $^{25}\text{Mg}$ ,  $^{65}\text{Cu}$  and  $^{67}\text{Zn}$ . The element uptakes are followed through analysis of isotope ratios in samples of ileum, faeces, urine and blood. The very small concentrations in the samples due to high-priced enriched isotopes and strong dilution make the investigation a real challenge to analytical methods. The project will continue for the next couple of years.

The widespread interest for better information of toxic elements in earth and water has created a stable contact to industry and environmental authorities for the use of ICP/MS to semiquantitative and screening analysis. The quick answer to the state of 25 or more elements in a single analysis is extremely useful and makes basis for the choice of precise analysis, if necessary.

ICP/MS is basically an instrument for trace element analysis, but it has proven important also in determining the stoichiometric composition of superconductors. The project has extended from the initial work with Y, Ba and Cu to involve e.g. rare earth elements bringing up difficult analytical problems easily solved by ICP/MS.

Ionchromatography (IC) has proved most suitable to determine anions in decontamination and migration experiments and in organic synthesis, and under certain conditions some cations are best determined this way.

In addition to obligations at Risø e.g. analysis of samples from the nuclear reactors DR1 and DR3 for evidence of corrosion, the presence of monitoring the U-concentration, analysis are car-



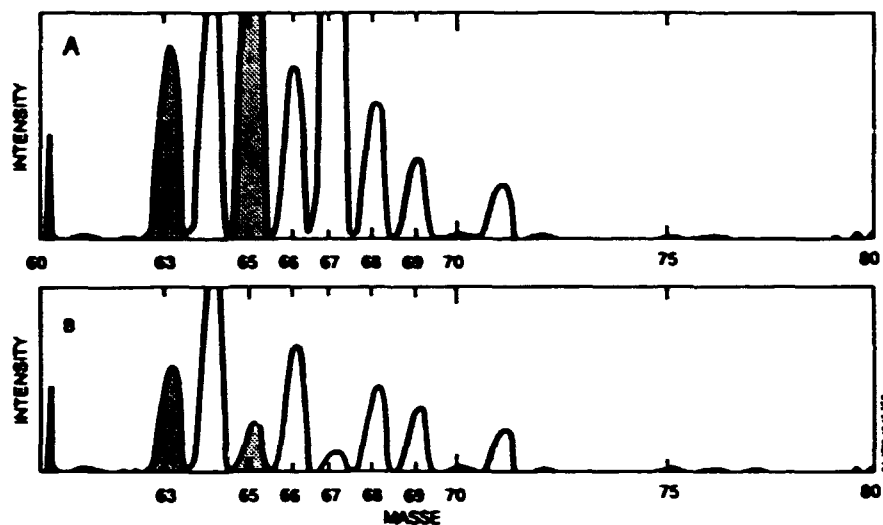


Fig. 5.4.1. Spectrum A is from the content of the ileum sampled immediately after a pig had been fed the enriched non-radioactive isotopes  $^{65}\text{Cu}$  and  $^{67}\text{Zn}$ . Spectrum B was samples 10 days later. There are distinct differences in the ratios between  $^{63}\text{Cu}/^{65}\text{Cu}$ ,  $^{66}\text{Zn}/^{67}\text{Zn}$  and  $^{68}\text{Zn}/^{67}\text{Zn}$ .

ried out for other laboratories and for the industry on a commercial basis.

## 5.5 Syntheses

The lack of commercially available chemical compounds often presents a hindrance in scientific research and for this reason, the chemistry section has also in 1990 carried out the most varied kinds of synthesis for customers from inside and outside Risø. The syntheses have so-far been in the laboratory scale, but a new pilot-scale laboratory for synthesis, located at the Risø Waste Management Section, is now ready for use.

A fair amount of the time in 1990 was spent on synthetic work carried out for a private firm, with the results becoming an important element in a patent application. Another, project on sensors for Radiometer Ltd. was concluded. This cooperation is expected to continue at a later stage. Metal complexes of the type tetra-aza-cyclo-tetradecan, various guanidin compounds and dihydroxydinaphtylsulfid have been synthesized for MODECS.

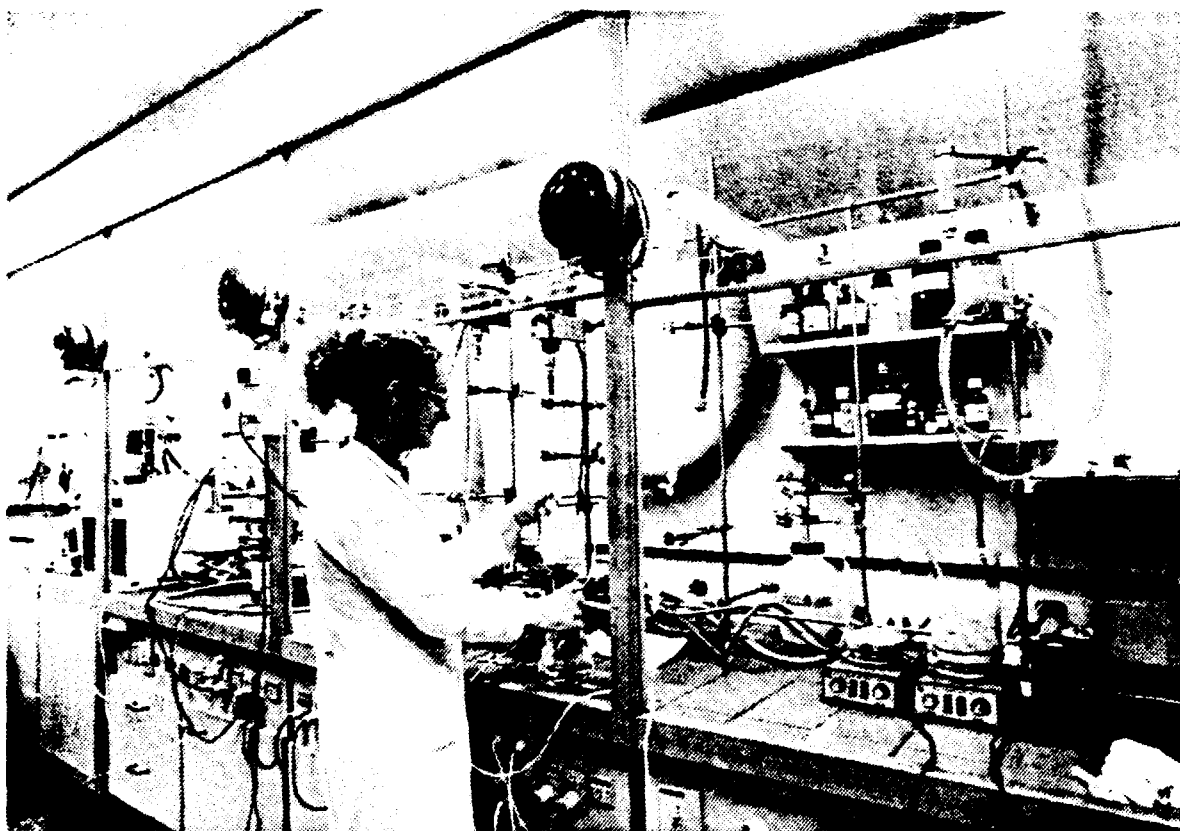
A substantial number of the syntheses in 1990 were carried out in connection with R&D projects at Risø.

ClO-radicals are generated from high-purity dichloroxid, which was synthesized for use in KER section in connection with a Ph.D.-thesis, the topic of which was the examination of dimerization velocities associated with studies in the ozone layer. Series of alkylnitrater, nitroalkener and nitrosoalkaner concerned with atmospheric chemistry were synthesized in 1990. The results of the kinetic experiments have been published in Chemical Physics Letters.

Methods for the synthesis of nitroacetamid and nitramid have been developed for the FBK section. The compounds are to be used in a collaboration with Tech. Univ. Berlin. The work is centred on studies of the parent nitrosamine,  $\text{H}_2\text{NNO}$ , the key-intermediate in the T<sup>1</sup>ermal DeNO<sub>x</sub> process. The synthesis of a suitable <sup>15</sup>N labelled precursor is in the planning in order to get a more detailed insight in the chemistry of the isolated  $\text{H}_2\text{NNO}$  molecule.

Another project in the FBK section aiming at the identification and quantization of allergy related matters, has led to the synthesis of a number of compounds e.g. tert.-butyl-hydroxybenzaldehyd.

Preliminary tests concerned with the development and possible future production of ceramic



*Fig. 5.5.1. The synthesis laboratory. A view.*

materials have been carried out for AFM in connection with their DK-SOFC cooperation project.

## 5.6 MODECS

Among the main achievements in 1990 has been the birth of **MODECS**, a club for industries and institutions interested in **MO**lecular **DE**sign of Chemical Systems. At present, 17 danish firms are members of the club and four institutions are attached.

**MODECS** is managed by a planning group, which held five meetings during 1990. Two joint sittings were arranged, and two newsletters have been distributed and a third is on its way. At the meeting in September the main subjects were

»Supramolecular Chemistry and Interface Structure« and »Interface Structures and Properties«, combined with an evening of discussion on research policy in theory and in practice. A joint sitting is planned at Risø on April, 1991 with the subjects »Surface Modification« and »Biocompatibility«.

The planning group is trying to identify one or more research areas of joint interest for possible attraction of industrial finances, and is considering whether **MODECS** ought to sub-finance certain joint projects. The matter will be discussed further at the first joint sitting in 1991.

The meetings have resulted in some agreements on cooperation with possible financial support from national research programs.

## 6 Ecology and Mineral Cycling

### 6.1 The MARINA Project

In 1985 the Commission of the European Communities invited the member states to participate in a project to evaluate the radiological impact of radioactivity in northern European marine waters. The name MARINA was adopted for the project which was chaired by Risø. The project was concluded at a seminar in Bruges 14-16 June 1989, where the results of the project were summarized and presented to a larger audience. The final report and seminar proceedings were published in 1990.

The radiological impact has been assessed in terms of radiation doses to humans in EC member states from exposure via marine pathways. The results show that the impact is by far dominated by the contribution from natural radionuclides for which the doses contribute more than 99% of the total. The second largest contribution is from civil nuclear site discharges which amount to about 0.3% of the total, followed by weapons test fallout and Chernobyl fallout of which each contributes less than 0.1%. In addition, the contribution from disposals of radioactive wastes at the North-east Atlantic dumpsite has been assessed at two orders of magnitude lower than that from civil nuclear site discharges.

The measured environmental data collected have been compared with model predictions. This comparison has shown that the water-circulation model describes with reasonable accuracy the dispersion of radionuclides in the European shelf seas. The predicted concentrations of the radionuclides considered were generally well within an order of magnitude of their measured values. Areas of poor correlation between predic-

ted and measured concentrations tended to be at some distances from the source, and often occur where uncertainties may exist in some of the model parameters. These parameters include water-transfer coefficients, sediment interactions and bioindicator concentration factors.

For the natural radionuclides the main contributor is the ingestion of  $^{210}\text{Po}$  in seafoods which accounts for about 80% of the collective dose. Around half of the  $^{210}\text{Po}$  contribution arises via mollusc ingestion. The major exposure pathway of the EC population from civil nuclear discharges is fish consumption and the most significant radionuclide is  $^{137}\text{Cs}$ .

### 6.2 »SENSI«: A Model Describing the Accumulation and Time - Integration in the Bioindicator *Fucus Vesiculosus* of Radioactive Discharges

In the process of designing countermeasures against anthropogenic effects on natural environments, time series of monitoring data are of central importance. All substitutes for actually measured time series, such as sophisticated prediction models, are subject to »wishful thinking« or »conservatism«. On the other hand, once one has access to well-documented monitoring data, modelling becomes a central tool in extracting general information from such data.

The SENSI model is designed to compare monthly monitoring data on radionuclide concentrations in a bioindicator sampled near a nu-

*Table 6.1.1. Collective exposure of the population of the European Community up to the year 2500 from radionuclides in northern European Waters*

Source	Collective dose to year 2500 (man Sv)
Civil nuclear site discharges	5300
Weapons test fallout	1600
Chernobyl fallout	1000
Sea dumping of solid radioactive waste	50
Natural radionuclides	1,700,000

# SENSI, Zn-65 Ringhals, 1983 - 1985

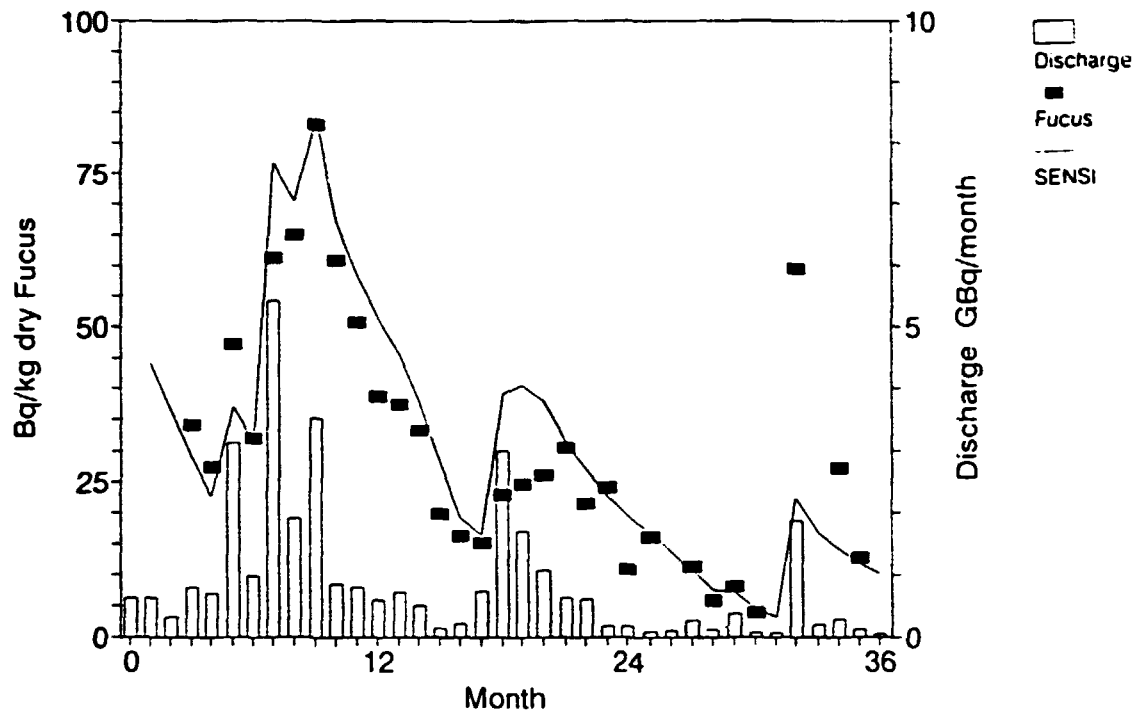


Fig. 6.2.1. Zn-65 discharges from Ringhals Nuclear Power Plant (bars) and calculated (SENSI curve) versus measured (■) concentrations in *Fucus vesiculosus* during 36 months January 1983 - December 1985.

clear power plant with calculated values based on reported discharges. The basic idea has been to include accessible parameter values of central importance to the measured concentrations and their variation. In the present work, these are relative accumulation rates describing the uptake, and physical decay, biological loss and bioindicator growth which affect a decrease in radionuclide concentrations. As each of these parameters can be measured separately, the model improves the knowledge of bioindicator functions and their limitations, and helps to identify biological parameters that should be studied further in order to improve the monitoring process.

The function and performance of the model are exemplified with monthly data on the brown algae *Fucus vesiculosus*, but the fundamental concept is equally valid in connection with other bioindicators and for other sampling intervals. *Fucus* is a much used bioindicator for nuclear discharges: it is easy to sample all year, it accumulates low metal and radionuclide water concentrations and it retains the accumulated elements long enough to enable a reasonable time

integration of fluctuating levels.

It can be concluded that

- *Fucus* is a valuable bioindicator in monitoring programmes because it integrates and concentrates low levels of metals and radionuclides,
- A dilution due to growth is more important than the actual loss of elements in determining the decrease in concentrations of several long-lived radionuclides and metals in *Fucus*,
- There is a need for studying biological factors such as biomass turnover rates and environmental parameter effects on pollutant accumulation and loss, when bioindicators are used in environmental monitoring programmes,
- The SENSI concept improves the use of *Fucus* as a bioindicator by including ecological factors and thereby increasing the correlation between expected and measured values.
- The SENSI model may be used to estimate the size of an uncontrolled discharge and to evaluate routinely the quality of discharge data. The present work indicates that dis-

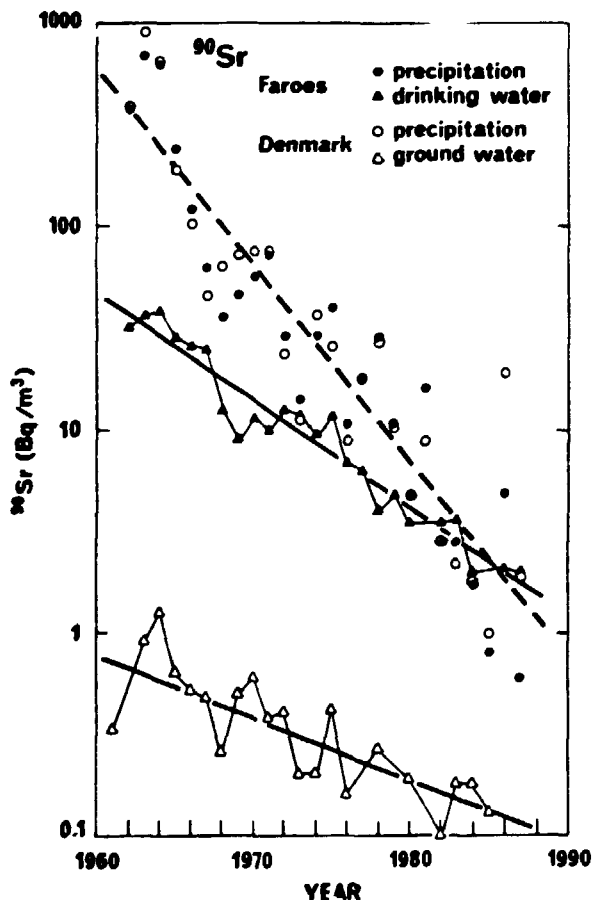
charge and to evaluate routinely the quality of discharge data. The present work indicates that discharge data from the nuclear power plant Ringhals are of a high quality.

### 6.3 Radioactive Contamination of Drinking Water

In the event of a nuclear war or a major nuclear accident, it is important to know to what extent there is a threat to the quality of drinking water. Is it necessary and possible to take any special precautions to protect this vital component of the human diet?

A comparison was made between Denmark, the Faroe Islands and Greenland of the contamination of water resources with strontium-90 from nuclear weapons fallout and cesium-137 from the nuclear accident at Chernobyl. It was found that Danish ground water used as drinking water was nearly totally protected by morainic surface soils. Faroese drinking water from streams that run along a peaty soil surface had a 30 times higher

Fig. 6.3.1. Strontium-90 measurements in various water resources presented as geometric means of values from individual sites.



contamination level and contributed about 3% to the total contamination of the human diet. The drinking water in Greenland was taken from streams that run along a morainic soil surface. It showed contamination levels that were 100 times higher than Denmark and contributed about 15% to the total contamination of the human diet during the last 10 years.

It could thus be concluded that it is only when contaminated surface water is used directly as drinking water that one need consider any protective measures towards the public.

### 6.4 Fish Ecotoxicology

The response to stress of aquatic organisms is characterized by its interaction with a series of environmental factors. When dealing with animals captured from the wild there is a risk of substantial biological variation at the year-to-year level.

In eels captured in Roskilde Fjord in 1972 and 1975 a specially enhanced synthesis was found in the gills from  $^{14}\text{C}$ -acetate of  $^{14}\text{C}$ -labelled mono-unsaturated fatty acids ( $\text{C}_{16:1}$  and  $\text{C}_{18:1}$ ) relative to saturated fatty acids ( $\text{C}_{16:0}$  and  $\text{C}_{18:0}$ ) in seawater, four days after irradiation (10 Gy,  $^{60}\text{Co}$ ). Corresponding experiments in 1976 and 1982 showed rather the opposite: irradiation resulted in more  $^{14}\text{C}$ -labelled saturated fatty acids relative to unsaturated fatty acids in the gills, both in fresh water and in seawater. The latter effect was less marked than in 1972 and 1975, but still statistically clearly significant.

Fig. 6.4.1. A blood sample is taken to assay changes in osmoregulation after copper poisoning of rainbow trout.



Such a lack of biological reproducibility may well represent a general trend that is more widespread than normally presumed. Nevertheless, it is the sublethal, quantifiable biological effects, such as described here, that are of special interest in ecotoxicology. One may simply have to accept that these often have to be assayed in long time series.

## 6.5 Radioecological Cooperation with the USSR

In May 1990 the Ecology Section participated in an international radioecological expedition to the USSR. Three areas with enhanced radioactivity levels were visited. The NW of the Black Sea, the Sverdlovsk area in the southern Urals and the Chernobyl reactor site in the Ukraine. We worked together with Soviet radioecologists at three locations and brought home a variety of interesting and unique samples.

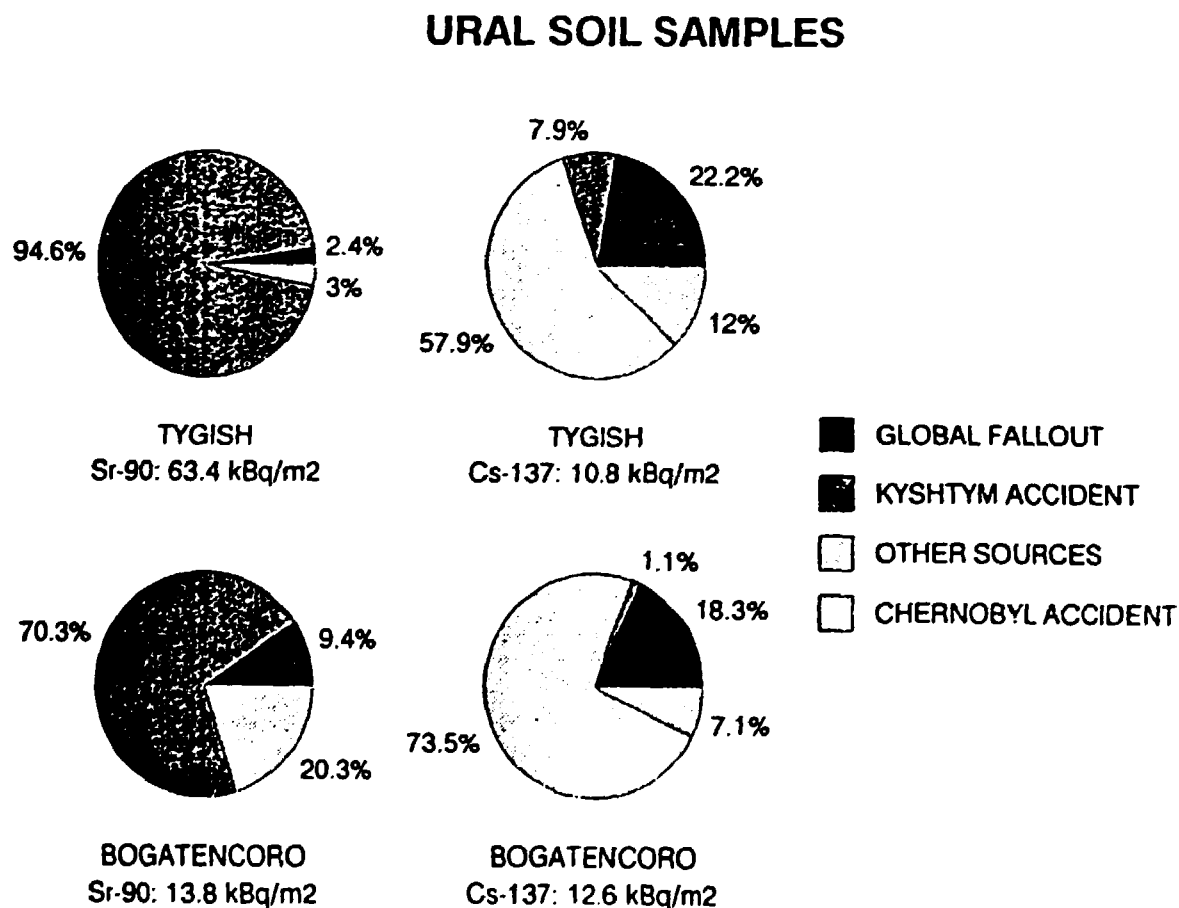
The most remarkable observations were made in the southern Urals. In this area at Kyshtym a

chemical explosion occurred in 1957 in a tank containing nitrate-acetate high-level radioactive waste. Radioactive fission products were deposited in parts of the Chelyabinsk, Sverdlovsk and Tyumen provinces. Among the long-lived fission products, the most prominent was Sr-90 of which about 2 PBq was released. Surprisingly little Cs-137 (~ 14 TBq) arose from this accident. The accident had been kept secret by the Soviet authorities until the late eighties.

The analysis of the Ural samples showed that the amounts of Cs-137 were incompatible with the expected levels arising from global fallout from the three sources: nuclear weapons testing, Chernobyl debris and the above-mentioned Kyshtym accident. Significantly more Cs-137 than had been expected from these sources were found in the samples.

In the last year an increasing amount of information on other sources of radioactive contamination in the southern Urals has appeared. Soviet scientists have thus mentioned an accident which took place in 1967, when a hot summer followed a dry winter. Some water evaporated from Lake

Fig. 6.5.1. (next page) The relative contribution from the four sources to contamination of soils in the Urals with strontium-90 and cesium-137.



Karachay, 15 km east of Kyshtym. This lake has been used for disposal of about 4 EBq Sr-90 and Cs-137. Dust from the lake bed was blown over a vast area, up to 75 km long. Some 20 PBq Cs-137 and Sr-90 from the shores of Lake Karachay contaminated about 1800 km<sup>2</sup>. This accident seems to explain the enhanced Cs-137 found in the environmental samples analysed at Riso.

A global perspective of the huge amounts of Sr-90 and Cs-137 disposed in fresh water systems of the southern Urals is the possibility of their

transfer by the Siberian rivers to the Arctic Ocean. Studies of the inventories of anthropogenic radionuclides in the oceans suggest that the Arctic Ocean contains significantly higher levels of Sr-90, Cs-134, Cs-137 and Pu-239, 240 than expected from the known sources, notably global fallout, waterborne discharges from nuclear reprocessing in western Europe and the Chernobyl accident. The surplus activity may have its origin in the southern Urals. (kBq : 10<sup>3</sup>Bq, TBq : 10<sup>12</sup>Bq, PBq : 10<sup>15</sup>Bq, EBq : 10<sup>18</sup>Bq).

## 7 Other Activities

### 7.1 Deposition and Removal of Radioactive Substances in an Urban Area

Until about 5 years ago, when studying off-site consequences of nuclear accidents by various groups of researchers, little or no account was taken of the special conditions encountered when the contamination reached urban areas. This was a remarkable oversight in view of the fact that the great majority of the population of Western Europe live in towns. Perhaps the reason was that virtually no input data were available to permit reasonable calculations and predictions.

The accident at Chernobyl in 1986 presented an opportunity to study the behaviour of radioactive fallout in the urban area. It appears that very few of the data applicable to a contaminated rural area are also valid for an urban area. Furthermore, the distribution pattern of fallout in an urban area is very dependent on whether the deposition took place in dry or wet weather.

It was found that the degree of interception of the fallout varies according to the type of urban surface and the physico-chemical form of the radionuclides. Only little iodine is retained on hard surfaces, while the retention of cesium and ruthenium is very significant on these surfaces.

In-situ measurements following wet deposition have shown that in the first few days after deposition about 60% of the cesium are removed from asphalt, concrete and granite pavements by runoff and street cleaning.

Dry deposition velocities of cesium to vertical

walls have been found to be very low, whereas they were 5-10 times greater on roads. On rough surfaces, such as corrugated roofs and grass, the deposition velocities are even higher.

Deposition indoors depends on the furnishing in the room. Deposition indoors has now been shown to be a relatively important source of radiation in houses that are well-shielded by their walls towards radiation from the outside.

People present in contaminated areas are shielded by various obstacles. The shielding factor, relative to the dose rate measured one meter above a grassed area can range from 0.5 to 10,000. The low figure refers to a case where trees and bushes are present. The highest figure should be used for well shielded basements. This range illustrates the importance of using accurate shielding factors in dose calculations.

The investigations have confirmed that a marked reduction in inhalation dose during the passage of a radioactive cloud can be achieved by remaining indoors. For modern Scandinavian houses, this reduction is about a factor of 3.

Using numerical values for the various parameters studied, it has been possible to illustrate how different surfaces contribute to dose rates to people present in an urban area. Generally, in the case of dry deposition, a garden with trees and bushes presents the main source of radiation. Roofs are also important, especially in an environment in which small houses are prevalent. In the case of wet deposition, the contribution to dose comes mainly from the ground (garden surface and street surface), but roofs are also important.



*Fig. 7.1.1. (next page) Collection of samples from radioactive areas in Gårle.*

## 7.2 The Raman Group

The work was mainly concentrated on studies of the potential energy surfaces of electronic excited states of organic molecules. Firstly, investigations of polyenes were extended into the region of low temperatures, to measurements of activation energies of triplet decay and to *cis-trans* photoisomerization studies in solution. Secondly, the photophysics of supramolecular compounds (crown-ethers) in solution were investigated. Thirdly, resonance Raman studies of keto-enol-enoltautomerism in excited electronic states were performed.

In connection with the first topic the collaboration with Dr. F.W.Langkilde (Pharmaceutical University, Copenhagen), Prof. G. Orlandi and F.Negri (Bologna, Italy) and Dr. A.M.Brouwer (Amsterdam, The Netherlands) was continued. Molecular systems studied experimentally and theoretically were model compounds of general interest such as stilbene and 1,3,5-hexatriene. A Ph.D. study (S.Møller in collaboration with Dr. F.Duus, Roskilde Univers-

ity) was continued. The second topic was pursued in a joint project between Cismi, University of Copenhagen (Prof. K. Bechgaard and A.M.Byrnard) and Radiometer Ltd. (Dr. N.-H.Jensen). Finally, the third project was a collaboration with the Polish Academy of Sciences.

## 7.3 Dosimetry and Radiation Processing

The calibration, measurement and consulting services have increased during 1990, as a consequence of several factors:

- There is an increased demand for measurements that are traceable to national standards in connection with the requirements derived from the implementation of the ISO-9000 standards on quality assurance.
- There is an increased use of radiation, particularly for sterilizing medical equipment.
- Several new accelerator facilities have been started or are under construction.

We have applied for authorization as a High



Dose Reference Laboratory according to the CEN-45001 standard for testing laboratories. The application, that is a consequence of the requirements from customers mentioned above, has been sent to the Danish Institute of Fundamental Metrology: if approved it will give official international recognition of the dosimetry work. It was required to write a quality manual that describes the procedures followed in the dosimetry work, and the way in which traceability of the measurements is obtained. The cooperation with National Physical Laboratory, Teddington, UK under a Euromet project provides the means for establishing traceability for the gamma and electron measurements, and during 1990 the procedures to be followed were developed.

In the fall a training course in dosimetry for industrial electron beam users was organized. Nine participants from Denmark, Sweden, Belgium and Japan followed the 2-week course.

Irradiation of materials on the 10 MeV electron accelerator has continued to grow in spite of the difficulties caused by technical problems at the accelerator. The beam power has been reduced to approximately 50% and the irradiations have therefore taken longer time, leaving less time for pulse radiolysis experiments. It is expected that the problems will be solved in the beginning of 1991.



*Fig. X. The set-up for radiation processing on the 10 MeV electron accelerator.*

## 8 Large Facilities

### 10 MeV Linear Electron Accelerator

The Risø linear accelerator was manufactured in 1975 by Haimson Research Corp. Electrons are accelerated by an electromagnetic microwave field with a frequency of 2856 MHz. The accelerator is used for radiation sterilization, plastic modification, food treatment, radiation hardness testing, semiconductor modification and initialization of chemical processes in kinetic investigations. Products are irradiated on a conveyor belt with electrons striking above. Maximum product size is normally  $80 \times 60 \times 30 \text{ cm}^3$  (l w h), but other sizes may be accommodated. The maximum height depends on the product density.

### Pilot Plant for Metal Extraction

A complete and self-contained pilot plant was established at Risø in 1981 initially to handle metal extraction under alkaline and neutral conditions from a variety of ore types and minerals.

The heart of the plant is a 1200 m long pipe reactor which makes possible continuous treatment of suspensions at 280°C and 100 bar. The pilot plant has since intermittently been used to implement our wet oxidation process on a semi-industrial scale.

### Experimental Farm

DYSKÆRGAARD is an experimental farm with approximately 120 hectares of arable land. The experimental farm is partly used for field experiments, and partly for grain and beef production.

## Open Top Chamber Facility

In collaboration with the National Environmental Research Institute and University of Copenhagen the Department operate a large Open Top Chamber facility situated at Risø. The facility consists of 19 large chambers (3.5 meters in

diameter) and 26 small chambers (1.5 meters in diameter). The small chambers have been equipped with lysimeters for water and nutrient balance studies, and for measuring nutrient leaching from the soil profiles. The chambers are used for short- and long-term studies of the effects of air pollution on crop plants and trees.

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- Gissel-Nielsen, G.* Agriculture, environment and society (in Danish). - Stevns Lokalforedragsforening, Nov 20, 1990.
- Gwozdz, R. and Kunzendorf, H.* Large-sample instrumental neutron activation analysis for environmental reconnaissance. Conference EUROANALYSIS VII, Vienna, 1990.
- Hansen Heinz, J.M.* Significant year-to-year variation of the response to radiation-induced stress shown by gill fatty acid metabolism in eels (*anguilla anguilla*) captured from the wild. 12th ESCPB-conference »Physiological and Biochemical Approaches to the Toxicological Assessment of Environmental Pollution«, Utrecht, August 27-31, 1990.
- Hansen, K., Jensen, P.K. and Kunzendorf, H.* The fission track method - a tool in Danish basin modelling. EAPG meeting Copenhagen, 1990.
- Hansen, K., Jensen, P.K. and Kunzendorf, H.* Temperature history in Danish sedimentary basins as revealed by the fission track method. 7th. Internat. Conference on Geochronology and Isotope Geology, Canberra, 1990.
- Jacob, P., Ertel, J., Paretzke, H.G. and Roed, J.* Deposition of radionuclides on trees, subsequent distribution processes and radiation exposure. First Internat. Conference »Biological Aspects of the Consequences of the Chernobyl Power Station Accident«, Zeleny Mys, USSR, Sep 10-14, 1990.
- Jakobsen, I.* Spread and P uptake of external hyphae differ between isolates of VA mycorrhizal fungi. - School of Agriculture, University of Western Australia, 18 Jul and Waite Agricultural Research Institute, University of Adelaide, Jul 30, 1990.
- Jakobsen, I., Abbott, L.K. and Robson, A.D.* Hyphal spread and P uptake differ between VAMycorrhizal fungi. - 8th North Amer. Conf. on Mycorrhizae, Jackson, Wyoming, Sep 5-8, 1990.
- Jakobsen, I.* The role of VA mycorrhiza in nutrient cycling. - Einsiedeln, Switzerland, First Conference in COST-Action 810, Oct 8-12, 1990.
- Jensen, E.S.* Agriculture and biological N<sub>2</sub> fixation in Denmark. - Rubber Research Institute Kuala Lumpur, Feb 2, 1990.
- Jensen, E.S.* Nitrogen fixation in field pea and the influence of pea cultivation on the turnover in soil (in Danish). - NJF-møde: Trindsådesodling, Uppsala, Sweden, Oct 16-17, 1990.
- Jensen, E.S. and Jensen, M.B.* Automated analysis of 15N and total N in plant material and soil. - Symp. on the Use of Stable Isotopes in Plant Nutrition, Soil Fertility and Environmental Studies, Vienna, Oct 1-5, 1990.
- Jensen, H.P. and Jørgensen, J.H.* Resistance genes to powdery mildew in new spring barley varieties and their distribution in Denmark 1977 to 1989. - Second European Workshop Integrated Control of Cereal Mildews: Virulence Patterns and Their Change, Roskilde Højskole, Jan 23-25, 1990.
- Jensen, J.* New high-yielding high lysine mutants in barley. - Int. Symp. The Contribution of Plant Mutation Breeding to Crop Improvement, Vienna, Jun 18-22, 1990.
- Jensen, P.K., Hansen, K. and Kunzendorf, H.* Basin modelling and annealing of fission tracks in the Danish area. Abstract. Conference on Geochronology and Isotope Geology, Canberra, 1990.
- Johansen, A.* The influence of VA mycorrhiza for turnover and transport of nitrogen in the root zone (in Danish). - Statens Seruminstitut, May 9, 1990.
- Johansen, A.* The metabolism of nitrogen in VAMycorrhiza. - Botanisches Institut der Universität Basel, Switzerland, Jun 19, 1990.
- Johansson, A., Rasmussen, H.N., Theilade, B. and Rasmussen, S.K.* Cloning of genes coding for barley peroxidases. - 2nd Int. Symp. on Molecular and Physiological Aspects of Plant Peroxidases, Lublin, Aug 27-29, 1990.
- Johansson, A. and Rasmussen, S.K.* Cloning of a cDNA for a barley peroxidase BP1. - 2nd Nordic Conf. on Protein Engineering, Helsingør, Oct 10-11, 1990.

- Johansson, A. and Rasmussen, S.K.* Cloning of a cDNA for the barley peroxidase BP1. - Biochemical Society's 20th Annual Meeting, Ebeltoft, Oct 25-27, 1990.
- Jørgensen, J.H.* Defence mechanisms of barley to powdery mildew (in Danish). - Institute for Crop Plant Genetics, Swedish Agricultural University, Svalöv, Sweden, Apr 2, 1990.
- Jørgensen, J.H.* Experiences from mutation studies on cereal disease resistance. - Int. Symp. The Contribution of Plant Mutation Breeding to Crop Improvement, Vienna, Austria, Jun 18-22, 1990.
- Jørgensen, J.H.* Mechanism of the Mlo resistance to barley powdery mildew. - James MacKey symposium, Genetics Centre, Swedish Agricultural University, Ultuna, Sweden, Nov 20, 1990. (Invited).
- Jørgensen, R.B.* Embryogenic single cells of barley. - Biotechnologisk Center for Planter, Annual Meeting, Carlsberg Laboratory, Jan 30, 1990.
- Jørgensen, R.B.* Effects and identification of conditioning factors in the medium of barley cell suspensions. - VII Int. Congress of Plant Tissue and Cell Culture, Amsterdam, The Netherlands, Jun 24-29, 1990.
- Kovacs, A., Metha, K. and Miller A.* »Characterization of linear electron accelerators using reference and routine dosimetry methods«. Int. Symposium on High-Dose Dosimetry for Radiation Processing. IAEA, Vienna, Austria, Nov 5-9, 1990.
- Kragh, K.* Chitinase in barley. - Biotechnologisk Center for Planter, Annual Meeting, Carlsberg Laboratory, Jan 29, 1990.
- Køie, B.* An embryospecific protein in barley. - Biotechnologisk Center for Planter, Annual Meeting, Carlsberg Laboratory, Jan 30, 1990.
- Langkilde, F.W. and Wilbrandt, R.* »Potentiale-nergiflader i excit erede triplet-tilstande af stilben og polyener«, invited lecture at the Annual Meeting of the Danish Chemical Society, Odense, Jun 7, 1990.
- Løgager, T., Holcman, J. and Sehested K.* »The oxidation of iron(II) by ozone in aqueous solution«. Xth Polish-Danish Symposium on Radiation Chemistry in Siedlce, Jun 23-27, 1990.
- Mathiasen, H., Larsen, E.T., Engell, K. and Køie, B.* Immunological and structural characterization of somatic embryogenesis in barley. - VII Int. Congress of Plant Tissue and Cell Culture, Amsterdam, Jun 24-29, 1990.
- Miller, A.* »Electron accelerators: Operation, Characterization, Dose Mapping, Routine Control«. IFFIT Training Course on Food Irradiation, Wageningen, Mar 1990. (Invited).
- Miller, A.* »EB-aktiviteter vid Risø forskningslaboratorium«. Electron Beam Seminarium, Åbo Akademi, Mar 8, 1990.
- Miller, A.* »Uncertainties in Radiation Processing Dosimetry«. IAEA Advisory Groups Meeting, Budapest, Apr 3-6, 1990.
- Miller, A.* »Radiation Processing Dosimetry«. Beijing Normal University, Beijing, May 18, 1990.
- Miller, A.* »Dosimetry and Process Control of Electron Beam and X-ray Irradiation«. Radia Industries, Takasaki, May 21, 1990. (Invited).
- Miller, A.* »Aspects of Radiation Processing Dosimetry«. Takasaki Radiation Chemistry Research Establishment, JAERI, Takasaki, May 22, 1990.
- Miller, A.* »A Reference Laboratory for Radiation Processing Dosimetry«. Danish-Polish Symposium, Siedlce, Jun 23-27, 1990.
- Miller, A.* »Maximum and Minimum Doses in Gamma and Electron Irradiated Products«. Medical Plastics '90, Malmö, Sep 11-13, 1990. (Invited).
- Miller, A. and Sharpe, P.H.G.* »Dichromate Dosimetry«. Euromet Workshop on Ionizing Radiation and Radioactivity. National Physical Laboratory, Teddington, Oct 7-10, 1990.
- Miller, A.* »The Role of Dosimetry in Process Control«. Int. Symposium on High-Dose Dosimetry for Radiation Processing. IAEA, Vienna, Nov 5-9, 1990.
- Munk, L., Jensen, H.P., and Jørgensen, J.H.* Virulence frequencies in barley powdery mildew in Eastern Denmark 1974-1986. - Second European Workshop Integrated Control of Cereal Mildews: Virulence Patterns and Their Change, Roskilde Højskole, Jan 23-25, 1990.
- Nielsen, K.* Establishment and biochemical characterization of embryogenic barley suspension culture. - Biotechnologisk Center for Planter, Annual Meeting, Carlsberg Laboratory, Jan 30, 1990.
- Nielsen, K.* Establishment and biochemical characterization of embryogenic cell suspensions of barley. - EMBO-workshop on Molecular basis of plant embryogenesis, Wageningen, Jun 15-19, 1990.

- Nielsen, K.* Identification and purification of chitinase and  $\beta$ -1,3-glucanase from embryogenic cell suspensions of barley. - VII Int. Congress of Plant Tissue and Cell Culture, Amsterdam, Jun 24-29, 1990.
- Nielsen, K.* Characterization of extracellular proteins from the medium of embryogenic cell suspensions of barley. - Glasgow University, Scotland, Aug 27-31, 1990.
- Nielsen, K., Kondrup, J., Stilling, B., Nielsen, K., Martinsen, L. and Jensen, E.S.* Protein turnover measured before and after oral hyperalimentation of malnourished patients with alcoholic liver cirrhosis. - ESPEN, Sep 16-19, 1990.
- Nielsen, O.J., Donlon, M., Sidebottom, H. and Treacy, J.* »Reactions of OH radicals with alkyl nitrates and nitroalkanes«. Eurotrac symposium '90 in Garmisch-Partenkirchen, Apr 2-5, 1990.
- Nielsen O.J., Donlon M., Sidebottom, H. and Jack, T.* »Reactions of OH radicals with alkyl nitrates and nitroalkanes«. Danish Chemical Society Annual Meeting, Odense, Jun 7, 1990.
- Nielsen, O.J.* »Gas phase reactions of OH radicals with organic nitrogen compounds«. Xth Polish-Danish Symposium on Radiation Chemistry in Siedlce, Jun 23-27, 1990.
- Nielsen, O.J., Donlon, M., Sidebottom, H. and Treacy, J.* »Kinetics and mechanisms for the reactions of hydroxyl radicals with alkyl nitrates and nitroalkanes«. The XIth International Symposium on Gas Kinetics in Assisi, Sep 2-7, 1990.
- Nielsen, O.J.* »Determination of rate constants for reactions with OH radicals - a method for testing HCFCs« (in Danish). Danish Center for Atmospheric Research Seminar at the University of Copenhagen, Oct 24, 1990.
- Nielsen, T.* Air pollution of PAH. Risø's and Copenhagen Environmental Surveillance Office's Air Pollution Seminar. Risø National Laboratory, May 17, 1990.
- Nielsen, T.* Measurements of polycyclic aromatic hydrocarbons (PAH). Danish Society of Environmental Chemistry's Meeting on »Special air pollution problems at an airport«. The Institute of H.C. Ørsted. University of Copenhagen, May 29, 1990.
- Nielsen, T.* Measurements and presence of nitro-PAH. Meeting of the DCAR Atmospheric Chemistry Working Group. Risø National Laboratory, Aug 13, 1990.
- Nielsen, T.* Occurrence of polycyclic aromatic hydrocarbons (PAH) at the international airport of Copenhagen. NOSA Aerosolsymposium. CTH, Göteborg, Nov 15-16, 1990.
- Nilsson, K.* Mechanisms and Interaction Phenomena Influencing Releases in Low- and Medium Level Waste Disposal Systems. 8. Progress meeting for Task 3.1 of the Third EC Programme on Radioactive Waste Management. Sellafield, GB, Mar 28-30, 1990.
- Pedersen, L.H.* 1,3- $\beta$ -D-glucansynthase activity and callose synthesis in barley Mlo mutants and mother varieties. - Bioteknologisk Center for Planter, Annual Meeting, Carlsberg Laboratory, Jan 29, 1990.
- Pedersen, L.H.* 1,3- $\beta$ -D-glucansynthase activity and callose synthesis in barley Mlo mutants and mother varieties. - 7th Congress of the Federation of European Societies of Plant Physiology, Umeå, Aug 5-10, 1990.
- Pedersen, L.H.* Synthesis of callose in barley leaves (in Danish). - Institute for Biochemistry and Nutrition, Technical University, 7 Nov and Institute of Plant Physiology, University of Copenhagen, Dec 12, 1990.
- Pilegaard, K.* 1990. Biological monitoring of heavy metal deposition. Seminar. National Center of Atmospheric Research (NCAR), Boulder, Colorado, Sep 27, 1990.
- Pilegaard, K.* 1990. Risø's Integrated Environmental Project (RIMI). AMS (American Meteorology Society) Symposium, Roskilde, May 2, 1990.
- Pilegaard, K.* 1990. The DCAR working group on effects. Yearly assembly of the Danish Centre for Atmospheric Research, Nov 27, 90.
- Rasmussen, H.N. and Rasmussen, S.K.* cDNA cloning of a barley leaf peroxidase. - Biochemical Society's 20th Annual Meeting, Ebeltoft, Oct 25-27, 1990.
- Rasmussen, M.* Genomic mildew DNA sequences expressed at an early stage of hyphal development. - Bioteknologisk Center for Planter, Annual Meeting, Carlsberg Laboratory, Jan 29, 1990.
- Rasmussen, M.* Cloning of genes for an obligate parasite *Erysiphe graminis* (in Danish). - Institute for Plant Biology, Royal Veterinary and Agricultural University, Copenhagen, Mar 14, 1990.
- Rasmussen, S.K.* Impact of biotechnology in agriculture (in Danish). - Kursus for frøavlere, Den Classenske Agerbrugsskole, Næsgård, Feb 5, 1990.

- Rasmussen, S.K.* Peroxidase genes in barley. - Institute for Plant Biology, Royal Veterinary and Agricultural University, Copenhagen, Feb 8, 1990.
- Rasmussen, S.K.* Expression of barley peroxidase genes. - Biotechnologisk Center for Planter, Annual Meeting, Carlsberg Laboratory, Jan 29, 1990.
- Rasmussen, S.K.* Expression of barley peroxidase genes. - 2nd Int. Symposium Molecular and Physiological Aspects of Plant Peroxidase, Lublin, Aug 27-29, 1990.
- Rasmussen, U.* Chitinase in *Brassica napus*. - Biotechnologisk Center for Planter, Annual Meeting, Carlsberg Laboratory, Jan 29, 1990.
- Rasmussen, U.* Immuno gold localization of antigens on plastic embedded plant material (in Danish). - Department of Biochemistry and Nutrition, Technical University of Denmark, Copenhagen, Mar 23, 1990.
- Rasmussen, U.* Molecular characterization of chitinase and their genes from pathogen-infected rapeseed (*Brassica napus* L. ssp. *deifera*). - Nordic Ph.D. course, Physiological Plant Pathology, Uppsala, Sep 12-19, 1990.
- Rosendahl, L.* Metabolic interactions between the legume host plant and nitrogen fixing bacteroids. - Australian National University, Canberra, Aug 2, 1990.
- Roed, J. and Goddard, A.J.H.* Ingress of Radioactive Material into Dwellings Presented at the Seminar on Methods and Codes for Assessing the off-site Consequences of Nuclear Accidents. May 7-11, 1990. 17 p.
- Roed, J. and Sandalls, J.* Decontamination in an Urban Area. Presented at the Seminar on Methods and Codes for Assessing the offsite Consequences of Nuclear Accidents, Athens, May 7-11, 1990.
- Roed, J.* The Skim and Burial Plough, USSR, Sep 4, 1990.
- Roed, J., Andersson, K. and Sandalls, J.* Reclamation of Nuclear Contaminated Urban Area. Presented at the BIOMOVs Symposium and Workshop on the Validity of Environmental Transfer Models, Stockholm, Oct. 8-12, 1990.
- Skou, J.P.* Inheritance of resistance to barley leaf stripe (in Danish). - Danish Society for Plant Pathology, Dec 11, 1990.
- Solar, S., Getoff, N., Sehested, K. and Holcman, J.* »Pulse radiolysis of carboxypyridines in aqueous solutions«. Xth Polish-Danish Symposium on Radiation Chemistry in Siedlce, Jun 23-27, 1990.
- Solgaard, P.* PlasmaQuad, ICP-MS (inductively coupled plasmamass spectrometry) for inorganic trace element analysis. Danish Society for Mass-spektrometry, May 30, 1990.
- Theilade, B.* Cloning and characterization of barley peroxidase genes. - Biotechnologisk Center for Planter, Annual Meeting, Carlsberg Laboratory, Jan 29, 1990.
- Thordal-Christensen, H., Cho, R.H., Brandt, J., Gregersen, P.L., Rasmussen, S.K., Smedegaard, V. and Collinge, D.B.* Cloning of sucrose synthetase, peroxidase and heat shock genes expressed amongst resistance response genes in barley induced by *Erysiphe graminis*. - Fourth Intern. Symp. on the Molecular Genetics of Plant-Microbe Interactions, Interlaken, Switzerland, Sep 9-14, 1990.
- Wilbrandt, R.* »Triplet states of polyenes: Experiment and theory«, Institut de Chimie Physique de l'Université de Fribourg, Oct 4, 1990.
- Øhlenschläger, M.* Differences in the Ability for Barley and Rye-grass Varieties to Absorb Caesium through the Roots. Dep. of Radiology and Radiation Biology, Colorado State University, Fort Collins, May 2, 1990.

# 10 Education

## 10.1 Ph.D. Projects

**Amstrup, B.**, 1990: »An ultraviolet resonance Raman intensity analysis of trans,cis,trans-1,3,5-hexatriene« Ph.D. Thesis, The Technical University, Lyngby.

**Christiansen, S.K.**, 1990: Establishment of restriction fragment length polymorphism (RFLP) analysis of the barley powdery mildew fungus (*Erysiphe graminis* f.sp. *hordei*) – Ph.D. Thesis, 59 p., Royal Veterinary and Agricultural University, Copenhagen.

**Kragh, K.M.**, 1990: Chitinases and  $\beta$ -1,3-glucanases in barley (*Hordeum vulgare* L.). – Ph.D. Thesis, 104 p., Royal Veterinary and Agricultural University, Copenhagen.

## 10.2 M.Sc. Projects

**Borbye, L.**: The use of pulsed-field gel electrophoresis in genome studies of *Erysiphe graminis* f.sp. *hordei* (in Danish). – M.Sc. thesis, Royal Veterinary and Agricultural University, Copenhagen, 32 p.

**Terkildsen, T. & Christensen, A.T.**: Extraction of Caesium from soil (in Danish). The Danish Engineering Academy, Lyngby.

## 10.3 Contribution to Scientific Courses

**Dahlggaard, H.**, IAEA International Training Course: Strategies and Methodologies for Applied Marine Radioactivity Studies, Santa Teresa, Italy and Monaco, Three lectures on monitoring in the marine environment: Monitoring general. Monitoring in Scandinavia and Monitoring after the Thule accident.

**Jakobsen, I.**, VA mycorrhiza and other endomycorrhizas - some general views, and: P acquisition in non-mycorrhizal and mycorrhizal plants. – Course arranged by the Universities of Copenhagen and Lund on »Soil Microbiology and Fertility«, Karlslunde.

**Miller, A.**, International training course in radiation dosimetry.

**Nielsen, O.J.**, External examiner at a Ph.D. dissertation at University College Dublin.

## 10.4 University Lectures

**Berenstein, D.**: Teacher at experimental courses in biochemistry and microbiology (Biology III), Roskilde University Centre.

**Jensen, E.S.**: Legumes:  $N_2$  fixation and residual value in the crop rotation (in Danish). – Lecture at the course Alternative farming, Royal Veterinary and Agricultural University, Copenhagen, Mar 7, 1990.

**Jørgensen, J.H.**: Three lectures at the course: Plant Breeding (in Danish), Royal Veterinary and Agricultural University, Copenhagen.

**Linde-Laursen, I.**: External tutor, Institute for Botany, Dendrology and Forest Genetics, Royal Veterinary and Agricultural University, Copenhagen.

**Nielsen, S.P.**, Lectures in radioecology, Technical University of Denmark.

**Røed, J.**, Lecture in atom physics and instruments, Technical University of Denmark.

## 11 Exchange of Scientists

- Anastasi, Dr. Christopher*, University of York, England.
- Andersen, K.G.*, Visiting scientists at UKAEA, Harwell, U.K.
- Broomfield, M.Mc. Mark*, University of York, England.
- Buxton, Dr. George*, University of Leeds, England.
- Carlson, Dr. Lena*, Marine Ecology Lund University, Sweden.
- Christensen, Dr. Hilbert*, Studsvik Energiteknik AB, Sweden.
- Chung, Dr. Woon H.*, Pusan, South Korea.
- Cruzen, Dr. Paul*, Max-Planck Institute for Chemistry, Mainz, Germany.
- Dowlen, Michael* Dublin Institute of Technology, Ireland.
- Genoff, Prof. Nikola*, University of Vienna, Austria.
- Gupta, Dr. B.L.*, Bhabha Atomic Research Centre, India.
- Hart, Dr. Ed J.*, Port Angeles, USA.
- Johansen, I.*, Visiting scientist at School of Agriculture, The University of Western Australia, Perth, until 15 Sep 1990.
- Joner, Stud.dr.scient. Erik*, Agricultural University of Norway, from 5 Nov 1990.
- Kovacs, Dr. Andreas*, Institute of Radioisotopes, Budapest, Hungaria.
- Loche, M.Mc. Garrett*, University College Dublin, Ireland.
- Mehra, Dr. Kishor*, AECL Whiteshell, Canada.
- Mordzinski, Dr. Andrzej*, Institute of Physical Chemistry, Polish Academy of Sciences, Poland.
- Newrym, M.Mc. Ruaidhri*, University College Dublin, Ireland.
- Polphong, Dr. Pornsri*, Office of Atomic Energy for Peace, Bangkok, Thailand.
- Ratajczak, Dr. Emil*, University of Wroclaw, Poland.
- Rosendahl, L.*, Visiting scientist at Murdoch University, Perth, Western Australia, until 15 Sep 1990.
- Sidebottom, Dr. Howard*, University College Dublin, Ireland.
- Sodeau, Dr. John*, University of East Anglia, Norwich, England.
- Solar, Dr. Sonja*, University of Vienna, Austria.
- Wallington, Dr. Tim*, Ford Motor Co., Dearborn, USA.
- Ohlenschlager, M.*, Visiting scientists at Colorado State University, USA.

## 12 Cooperative Projects

Radioactive tracers in the Greenland Sea. Part of the international Greenland Sea Project. *H. Dahlgaard*.

Studies on the Transport of Coastal Water from the English Channel to the Baltic Sea using Radioactive Tracers. Marine Science and Technology Programme (MAST), CEC. Cooperative project including Risø National Laboratory; Federal Maritime and Hydrographic Agency, Germany (Formerly DHI); Netherlands Energy Research Foundation; Commissariat à l'Energie Atomique, France; Ministry of Agriculture, Fisheries and Food, Lowestoft, UK. Project coordinator. 1990 - 1993. *Henning Dahlgaard, S.P. Nielsen*.

*Fucus* and *Mytilus* as bioindicators in monitoring for coastal metal and radionuclide pollution. Collaborator: IAEA, Monaco Laboratorium. *Henning Dahlgaard*.

Radioecology of seminatural ecosystems (CEC). Cooperative project including Risø National Laboratory, (DK), Nuclear Energy Board (IR) (Coordination), Institute of Terrestrial Ecology, Merlewood (UK), Swedish University of Agricultural Science, Uppsala (S), University of Thessaloniki (G). *Asker Aarkrog, S.P. Nielsen*.

EC-project on Deposition in Urban Areas. Collaborators: UKAEA, Harwell, GSF, München. *Jørn Røed*.

EC-project on Urban Decontamination. Collaborators: UKAEA Harwell, Catholic University, Leuven, Glasgow University. *Jørn Roed.*

EC-project on Indoor Deposition. Collaborator: Imperial College. *Jørn Roed.*

EC-project on Weathering and Resuspension. Collaborators: UKAEA, Colchester, GSF, München. *Jørn Roed.*

EC-project on Development of Skim and Burial Plough. Part of RESSAC; Main Collaborator Cadarache France. *Jørn Roed.*

NKA-project on Deposition and Removal of Radioactive Substances in an Urban Area. Collaborators: IFE Norway, Studsvik, Sweden. *Jørn Roed.*

NKA-project on Waste Created by Clean Up of an Urban Area. Collaborators: Helsinki University, Agricultural University, Oslo, SSI Sweden. *Jørn Roed.*

Radon in Cellars. Collaborators: Danmarks Teknologiske Institut. *Jørn Roed.*

IAEA project on Validation of Modul Parameters VAMP. *Jørn Roed.*

Risø's integrated environmental project (RIMI). *Kim Pilegaard.*

EUROTRAC-TOR Tropospheric Ozone Research. *Kim Pilegaard.*

EUROTRAC-FATE Formation of Aerosol and their transformation over Europe. *Kim Pilegaard.*

Uptake of Nitrogen compounds by above ground plant parts. Collaborator: National Environmental Research Institute DK. *Kim Pilegaard.*

Biological monitoring of heavy metals. Collaborator: Laboratory of Environmental Research Institute. Science and Education, The Technical University, Lyngby. *Kim Pilegaard.*

EUROMET Collaboration between European Metrology Laboratories. *Arne Miller.*

EUROTRAC - LACTOZ Laboratory Studies of chemistry related to tropospheric ozone. *Ole John Nielsen.*

IAEA Coordinated Research Programme on Dosimetry for Charged Particles. *Arne Miller.*

Danish Centre for Atmospheric Research (DCAR) – working group on atmospheric chemistry *Kim Pilegaard & Arne Jensen.*

NATO Collaborative research grant. *Denmark, Italy, The Netherlands.*

Evaluating the role of legume cover crops in the nitrogen nutrition of rubber. 1989-1993. EEC project under The International Scientific Cooperation Programme. Participants, Malaysia: M.N. Sudin, A. Ikram, A.W. Mahmud, The Rubber Research Institute of Malaysia, Kuala Lumpur. *I. Jakobsen and E.S. Jensen.*

Kombineret halmnedmuldning og efterafgrøde-  
dyrkning 1989-1991 (Combined straw incorporation and cultivation of catch crops 1989-1991). Participants: Ingrid Thomsen og Bent T. Christensen, Government Research Station Askov, Danish Research Service for Plant and Soil Science. *E.S. Jensen.*

Mikrobielle processer i rodzonen i relation til planternes forsyning med næringsstoffer 1988-1991 (Microbial processes in the root zone in relation to supply of plants with nutrients). Participants: Seven Danish research laboratories. *I. Jakobsen and A. Johansen.*

Hyphal phosphorus uptake by VA mycorrhizas; comparison of different fungal species. Participants: L.K. Abbott and A.D. Robson, The University of Western Australia. *I. Jakobsen.*

Exchange of metabolites across the peribacteroid membrane in legume root nodules 1989-1992. Participants: M.J. Dilworth and A.R. Glenn, Murdoch University, Western Australia. *L. Rosendahl.*

## 13 Guest Lectures

*Bowles, Dianna J.*, Biochemistry Department, The University of Leeds, England: »Cell signalling events in plant defense responses«.

*Buxton, George*, University of Leeds, England. »Free radical induced oxidation of sulphur(IV) in aqueous solution«.

*Crutzen, Paul*, Max-Planck Institute for Chemistry, Mainz, Germany. »Human activities and their impact on global atmospheric chemistry and climate«.

*Islam, M.R.*, Waite Agricultural Research Institute, Glen Osmond, South Australia: »Present status of genetics of rust resistance in flax«.

*Kunoh, Hitoshi*, Mie University, Tsu City, Japan: »Recognition of a fungal non-pathogen by barley coleoptile cells at the prepenetration stage«.

*Künzel, G.*, Zentralinstitut für Genetik, Gatersleben, Germany: »Cytogenetic studies based on reciprocal translocation in barley«.

*Mordzinski, Andrzej*, Institute of Physical Chemistry, Polish Academy of Sciences »Proton transfer spectroscopy of some selected molecular systems«.

*Palmgren, Michael*, Plantbiochemistry Department, University of Lund, Sweden: »Plasma-membrane + -ATPase from higher plants«.

*Sodeau, John*, University of East Anglia, Norwich, England. »Atmospheric Cryochemistry«.

*Wallington, Tim*, Ford Motor Co., Dearborn, USA. »Atmospheric chemistry and Ford Motor Co.«.

*Wallington, Tim*, Ford Motor Co., Dearborn, USA. »The atmospheric chemistry of ether fuel additives«.

*Warembourg, F.*, CNRS-CEPE, Montpellier Cedex, Frankrig: »Isotopes, powerful tools for understanding time dependent events occurring in the rhizosphere of plants«.

*Wellings, Colin R.*, Plant Breeding Institute, University of Sydney, Australia: »Pathotype evolution and epidemiology of stripe rust in Australia«.



# 14 Committee Membership

## 14.1 National

- A. Aarkrog:* Danish Contact Forum for Health Physics.
- A. Aarkrog:* Danish National Council for Oceanology.
- A. Aarkrog:* Danish reference group for CEC's MAST programme.
- A. Aarkrog:* Danish reference group for CEC's Radiation protection programme.
- A.J. Andersen:* Member of the board of Pajbjerg-fonden.
- H. Dahlgaard:* Greenland Sea Project, Danish working group.
- H. Doll:* Center leader for Bioteknologisk Center for Planter.
- H. Giese:* Member of SJVF; Bestyrelsen for Det Forstlige Forsøgsvæsen; Miljøfagligt Udvalg i Danmarks Naturfredningsforening.
- G. Gissel-Nielsen:* Member of SJVF; ATVs Akademiråd; Bestyrelsen for Statens Planteavlssøg; Miljøfagligt Udvalg i Danmarks Naturfredningsforening.
- B. Skytte Jensen:* Member of the Lignocellulose committee.
- J.H. Jørgensen:* Dansk Genbanknævn; Koordineringsgruppen for resistens og virulens i korn og kornsygdomme.
- Ole John Nielsen:* The National Committee for The International Geosphere-Biosphere Programme (IGBP).
- T. Nielsen:* Working groups on Atmospheric Research and Transport and Diffusion within Danish Center of Atmospheric Research (DCAR).
- K. Pilegaard:* Danish Centre for Atmospheric Research (DCAR) Working group on effects. (Chairmanship).
- P. Solgaard:* Dansk Standardiserings Råd. S168-Vandundersøgelse, FaU 13.

## 14.2 International

- A. Aarkrog:* IAEA expertgroup on Technical Report on the Assessment and Control of Radionuclide Discharges to coastal waters.
- A. Aarkrog:* IAEA CRP MARDOS (Chairman).
- A. Aarkrog:* IAEA VAMP programme (Consultant).

- A. Aarkrog:* International Union of Radioecologist (IUR) (Presidency until Oct. 1990).
- A. Aarkrog:* Programme Committee for CEC-IUR Seminar on Comparative Assessment of the Environmental Impact of Radionuclide Released During Three Major Nuclear Accidents in Kyshtym, Windscale, Chernobyl (Oct. 1990).
- A. Aarkrog:* CGC for CEC's Radiation Protection Programme.
- A. Aarkrog:* Programme Committee for Symposium and Workshop on the Validity of Environmental Transfer Models (BIOMOVs) (Oct. 1990).
- A. Aarkrog:* NEA/CRESP (member of executive group)(OECD).
- A. Aarkrog:* Reference group NKS-RAD programme.
- A. Aarkrog:* Group to evaluate the Research programme on Radioactive Fallout of the Norwegian Agricultural Research Council.
- A. Aarkrog, J. Roed:* SCOPE RADPATH group.
- A. Aarkrog:* Editorial Board of J. Environ. Radioactivity.
- H. Dahlgaard:* Baltic Marine Environment Protection Commission, Helsinki Commission (HELCOM). Group of Experts on Monitoring of Radioactive Substances in the Baltic Sea (MORS).
- H. Dahlgaard:* Marine Radioecology Working Group (MARECO), International Union of Radioecologists (IUR). (Working Group Leader).
- H. Dahlgaard:* Working group on a Nordic participation in the World Ocean Circulation Experiment. (WOCE).
- H. Dahlgaard:* Nordic Nuclear Safety Project (NKS), Radioecology programme (RAD) (Coordinator).
- H. Doll:* Executive committee, 6th International Barley Genetics Symposium, 1991.
- K.C. Engvild:* Member of Journal Committee of 'Physiological Plantarum'.
- H. Giese:* Executive committee, 6th International Barley Genetics Symposium, 1991.
- G. Gissel-Nielsen:* OECD committee for plant/soil/microbial interactions.

- I. Jakobsen:** Management committee for COST Action 810: The role of VA mycorrhizae in transformation of matter in the soil and their importance for plant nutrition and plant health.
- E.S. Jensen:** Editorial Board of 'Plant and Soil'.
- J. Jensen:** International coordinator, barley chromosome 5; International Barley Nomenclature Committee.
- B. Skytte Jensen:** Working Groups within the EEC R & D Programme Management and Storage of Radioactive Waste, MIRAGE, in the subcommittees CHEMVAL, COCO, and Natural Analogues. Member of WP Geological Disposal of Radioactive Waste. The Editorial Board of »Waste Management«.
- J.H. Jørgensen:** Editorial board of 'Euphytica'; International coordinator, disease and pest resistance genes; Working group on cereal disease resistance biology (Chairman), Scandinavian Association of Agricultural Scientists; Working group cereal (Chairman), Nordic Gene Bank; Working group barley, European Coordinated Programme Genetic Resources.
- H. Kunzendorf:** The Editorial Board of »Marine Mining«. Member of the Board of the »International Marine Minerals Society«. Member of its awards-committee in 1990. Member of the planning group for a new Technological Center for Marine Minerals. Reviewer of Marine Chemistry, Marine Geology, Marine Mining, Mineralium Deposita.
- I. Linde-Laursen:** Organizing Committee, TRITI-CEAE, an international symposium, 1991.
- Arne Miller:** ASTM Subcommittee on Dosimetry for Radiation Processing, E10.01.
- Ole John Nielsen:** COST 611 Concertation Committee on Physico-Chemical Behaviour of Atmospheric Pollutants.
- Ole John Nielsen:** COST 611 working party 2 Steering Committee, Atmospheric Chemical and Photochemical Processes.
- S.P. Nielsen:** International Committee on Radionuclide Metrology.
- S.P. Nielsen:** The Group on in-situ measurements (ICRU).
- T. Nielsen:** Member of the Board of Nordic Society of Aerosols (NOSA).
- K. Nilsson:** Third EC Programme on Radioactive Waste Management, Task 3.1 on: Characterization of Low- and Medium-Level Waste Forms.
- J. Roed:** IAEA and EEC VAMP Project Urban Group (Chairman).
- J. Roed:** The GRECA Group (OECD).
- J. Roed:** The Fuel Cycle Safety Group (OECD).
- J. Roed:** The MARIA Project (EEC).
- J.P. Skou:** Section for Plant Protection (Chairman), Scandinavian Association of Agricultural Scientists.

## 15 Seminars Organized

Second European Workshop, »Integrated Control of Cereal Mildew: Virulence Patterns and Their Change«, Roskilde, Denmark, 23-25 Jan 1990 (**J. Helms Jørgensen**).

# 16 Personnel

*Head of Department*  
Arne Jensen

## 16.1 Scientific Staff

Aarkrog, Asker  
Andersen, Arna J.  
Andersen, Lars (until 28.02)  
Berenstein, Dvora  
Bjergbakke, Erling  
Bjerre, Anne Belinda  
Christiansen, Solveig Krogh (from 01.04)  
Dahlgaard, Henning  
Doll, Hans  
Engvild, Kjeld C.  
Fenger, Jørgen  
Giese, Henriette  
Gissel-Nielsen, Gunnar  
Haahr, Vagner  
Hansen, Heinz J.M.  
Hansen, Knud Bent  
Holcman, Jerzy  
Holme, Inger (from 01.10)  
Jakobsen, Iver  
Jensen, Bror Skytte  
Jensen, Erik Steen  
Jensen, Hans Peter  
Jensen, Jens  
Jensen, Jørgen (until 1/12)  
Jørgensen, Jørgen Helms  
Jørgensen, Rikke Bagger  
Knudsen, Inge (until 30.04)  
Kunzendorf, Helmer  
Køie, Bertel  
Larsen, Else Toftdahl  
Linde-Laursen, Ib  
Lynggaard, Bent  
Mathiassen, Henrik (from 15.01)  
Miller, Arne  
Nielsen, Ole John  
Nielsen, Sven P.  
Nielsen, Torben  
Nilsson, Karen  
Pagsberg, Palle  
Pilegaard, Kim  
Rasmussen, Søren  
Roed, Jørn  
Rosendahl, Lis  
Rossen, Lone (until 31.01)

Sehested, Knud  
Sillesen, Alfred  
Skou, Jens Peder  
Solgaard, Per  
Sørensen, Emil  
Wilbrandt, Robert

## 16.2 Technical Staff

Andersen, Esther  
Baade-Pedersen, Pearl  
Bitsch, Gunnar  
Brandstrup, Oda  
Christensen, Tove  
Clausen, Jytte Lene  
Ebling, Elise  
Hansen, Elly  
Holm-Pedersen, Anna  
Hougaard, Henrik  
Jensen, Karen Mandrup  
Jørgensen, Vibeke  
Kjølhede, Alice  
Larsen, Ingelis  
Mundt, Helge Chr.  
Nielsen, Jette Bruun  
Nielsen, Karen Wie  
Prip, Henrik

Corfitzen, Hanne  
Hansen, Carl  
Johansen, Torben  
Kristiansen, Bo  
Larsen, Erik E.  
Larsen, Fritz  
Madsen, Michael  
Munk, Jette  
Nielsen, Svend

Andersen, Bente  
Andersen, Lis Brandt  
Andersen, Margit Elm  
Bendtsen, Bente (01.03-30.09)  
Brink Jensen, Merete  
Bruun, Henrik (01.04-31.10)  
Djurdjevic, Stanko  
Dyrgaard Jensen, Lone  
Gade, Poul  
Hansen, Ina  
Hasselbach, Finn

Henriksen, Ebbe  
 Holm-Jensen, Anne Grethe  
 Ibsen, Elly  
 Johansen, Hanne Bay  
 Karlsen, Aage  
 Larsen, Hanne Egerup  
 Larsen, Inge Merete  
 Lemée, Beth (until 31.01)  
 Lilholt, Ulla  
 Meltofte, Liselotte  
 Nielsen, Vagn Aage  
 Olsen, Anette  
 Olsen, Inge (from 08.10)  
 Poulsen, Aksel  
 Rasmussen, Charlotte  
 Sillesen, Anerikke  
 Skovsgaard, Bent  
 Steensen, Randi (until 30.05)  
 Thomsen, Jørgen D.  
 Vestesen, Hans

Carstensen, Jonna (1/6-5/10)  
 Fernqvist, Tomas  
 Green, Jytte  
 Jensen, Ellen Møller  
 Jensen, Hanne  
 Jørgensen, Ole  
 Lindskou, Fini  
 Olsen, Svend K.  
 Petersen, Susanne  
 Sørensen, Poul  
 Vinther, Lis  
 Vinther, Niels  
 Voss, Hanne (until 31/8)

### 16.3 Office Staff

Kornerup, Berit  
 Kristensen, Ingrid  
 Nielsen, Margit

Andersen, Annie  
 Madsen, Ruth

Frandsen, Anette (until 31.10)  
 Koch, Tonny (from 26.11)  
 Petersen, Lis  
 Sørensen, Anni

Bay, Kirsten  
 Jacobsen, Inger  
 Krogh, Helle  
 Pedersen, Ingrid (until 15/8)  
 Larsen, Åse Neve

### 16.4 Ph.D. Students

Ellermann, Thomas  
 Løgager, Tine  
 Markert, Frank  
 Møller, Søren

Andersson, Kasper Grann  
 Bertelsen, Finn  
 Borbye, Lisbeth  
 Christiansen, Solveig Krogh (until 30.04)  
 Johansen, Anders  
 Kragh, Karsten M.  
 Nielsen, Kirsten (until 31.07)  
 Olsen, Allan Gylling  
 Pedersen, Lars H.  
 Petersen, Lene  
 Rasmussen, Merete  
 Rasmussen, Ulla  
 Sørensen, Peter (from 01.03)  
 Theilade, Bodil  
 Øhlenschläger, Mette

### 16.5 M.Sc. Students

Borbye, Lisbeth  
 Johansson, Annette  
 Rasmussen, Henrik Nørgaard

### 16.6 Apprentices

Korgaard, Charlotte

Hansen, Nikolaj (01.01.-31.12)  
 Hansen, Trine (01.11)  
 Hansen, Tina Klarskov (01.02-31.05)  
 Jensen, Ulla Damm (from 16.11)  
 Larsen, Hans Peter (01.04-15.09)  
 Poulsen, Karin (01.06-05.08)  
 Risager, Kirsten (until 14.02))  
 Stormly, Lotte (01.01-31.05)  
 Tung, Tran Duc (from 01.09)

# 17 Acronyms

**BIOMOVS:** Biospheric Model Validation Study.  
**CEC:** Commission of European Communities.  
**CGC:** Management and Coordination Advisory Committee.  
**CRESP:** Coordinated Research and Environmental Surveillance Programme (under NEA).  
**CRP:** Coordinated Research Programme.  
**DCAR:** Danish Centre for Atmospheric Research.  
**DIA:** The Danish Engineering Academy.  
**EC:** European Communities.  
**EEC:** European Economic Communities.  
**EUROTRAC:** European Experiment on Transport and Transformation of Environmental Relevant Trace Constituents of Anthropogenic and Natural Origin.  
**FATE:** Formation of Aerosol and their Transformation over Europe (under EUROTRAC).  
**GRECA:** Group of Experts on accident Consequences (under NEA, OECD).  
**GSF:** Gesellschaft für Strahlen- und Umweltforschung.  
**GSP:** Greenland Sea Project.  
**HELCOM:** Helsinki Commission.  
**IAEA:** International Atomic Energy Agency.  
**IBSS:** Institute of Biology of the Southern Seas (USSR).  
**ICRM:** International Committee on Radionuclide Metrology.  
**ICRU:** International Commission on Radiological Units.  
**IFE:** Institute for Energy Technology Norway.  
**ICSU:** International Council of Scientific Unions.  
**IUR:** International Union of Radioecologists.  
**IVL:** Swedish Environmental Research Institute.  
**MARDOS:** IAEA CRP on «Sources of Radioac-

tivity in the Marine Environment and their Relative Contributors to Overall Dose Assessment from Marine Radioactivity».  
**MARECO:** Marine Radioecology Working Group (under IUR).  
**MARIA:** Methods for Assessing the Radiological Impact of Accidents. (Research programme under CEC).  
**MAST:** Marine Science and Technology Programme (under CEC).  
**MORS:** Group of Experts Monitoring of Radioactive Substances in the Baltic Sea.  
**NEA:** Nuclear Energy Agency (under OECD).  
**NKA:** Nordic Liason Committee for Atomic Energy.  
**NKS:** Nordic Nuclear Safety Project.  
**NLVF:** Norwegian Agricultural Research Council.  
**OECD:** Organisation for Economic Cooperation and Development.  
**RAD:** Radioecology Programme (under NKS).  
**RADPATH:** Biochemical Pathways of Artificial Radionuclides (under SCOPE).  
**RESSAC:** The Consequence of a Major Nuclear Accident on the Surrounding Environment: Soil and Surface Rehabilitation (under CEC).  
**RIMI:** Risø Integrated Environmental Project.  
**SCOPE:** Scientific Committee on Problems of the Environment.  
**TOR:** Tropospheric Ozone Research (under EUROTRAC).  
**UKAEA:** United Kingdom Atomic Energy Authorities.  
**VAMP:** Validation of Model Project (under IAEA).  
**WOCE:** World Ocean Circulation Experiment.

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Selected activities of the Environmental Science and Technology Department during 1990 are presented. The research in the department is predominantly experimental, and the research topics emphasized are introduced and reviewed in eight chapters: 1. Introduction, 2. The Atmospheric Environment, 3. Plant Genetics and Biology, 4. Nutrient Efficiency in Plant Production, 5. Chemistry of the Geosphere, 6. Ecology and Mineral Cycling, 7. Other Activities, 8. Large Facilities.

The department's contribution to national and international collaborative research programmes is presented together with information about large facilities managed and used by the department as well as activities within education and training.

Lists of scientific and technical staff members, visiting scientists, Ph.D. students, publications, lectures and poster presentations are included in the report.

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